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TFG

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INCREASING REFERENCE DATABASES FOR DNA BARCODING AND METABARCODING OF MARINE FISH

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ABSTRACT

Current methods for biomonitoring marine environments are aggressive, costly and time-consuming. DNA barcoding and metabarcoding offers a more accurate solution to this framework, but for this to be implemented, reliable reference sequences need to be available. The present study aimed to complete the reference database for metabarcoding studies. From an original list of 141 marine fish individuals, a total of 50 fish species were barcoded by PCR amplification of the 12s rRNA gene and sanger sequencing. A genetic analysis was additionally performed. Sequences are submitted to public access. Overall, DNA barcoding has shown to be a means by which marine resources could be surveyed without causing any negative impact.

Keywords: *DNA barcoding, DNA metabarcoding, marine fish, 12s rRNA, biomonitoring.*

INTRODUCTION

Current limitations on biomonitoring

Welfare of aquatic ecosystems is of major importance to human well-being. As human impacts continue to grow, so does the pressure to observe biodiversity. Large scale human activities such as overexploitation and pollution are causing irreversible environmental degradation. The daunting challenge that represents for marine ecosystems monitoring will require a revolution in monitoring technologies (Baird and Hajibabaei, 2012).

Characterization of biodiversity has been extensively used to confidently monitor and assess environmental status (Aylagas *et al.*, 2016). Current methods for such means mostly rely on biomonitoring based on morphological identification of taxonomic groups (fish, macroinvertebrates, meiofauna, etc.). Sampling and sorting of targeted organism are needed for this monitoring approaches. For instance, traditional methods for capturing fish include trawling, netting, electro-fishing etc. Hence, this *modus opperandi* has proven to be invasive, costly and time-consuming (table 1). Taxonomic identification also requires personal expertise and large sampling. Therefore, there is an urgent need to improve on strategies for both, biomonitoring and assessment of marine diversity.

Table 1. Properties of conventional methods employed for environmental monitoring (Pawlowski *et al*, 2020).

Time per sample	Fixed.
Costs per sample	Fixed.
Sensitivity	Generally low, requires large sampling efforts to obtain complete species list.
Taxonomic range	Limited to taxa that can be distinguished morphologically.
Detectability	Require intensive sampling.
sampling	Invasive.
Sample processing	Simple but manual.
contamination	Low risk.
infrastructure	Simple equipment needed.
Species identification	Based on personal taxonomic expertise and available literature.
Data analysis	Simple statistical tools.
Data interpretation	Depends on personal expertise and established ecological knowledge.

In contrast to conventional survey methods, methods based on genetics are being commonly used to identify specimens and the approach has wide applications in biodiversity conservation, environmental management, invasion biology, the study of trophic interactions, and food safety. DNA-based species identification, known as barcoding, transformed the traditional approach to the study of biodiversity science (Cristescu, 2014). This idea introduced by Arnot *et al*. (1988) and was firmly advanced and standardized by Hebert *et al*. (2003).

DNA Barcoding and metabarcoding

DNA barcode concept was proposed in 2003 by Herbert et al. They established that the mitochondrial gene cytochrome *c* oxidase I (COI) could serve as a global identification system for animals. Indeed, this is the most frequently employed gene for metazoans. Later studies revealed that rRNA genes worked for many marine groups (Vollmer & Palumbi 2004; Calderón *et al*. 2006).

DNA molecules contain genetic information specific to each species (Pawlowski *et al.*, 2020). Thus, DNA barcoding enables species recognition throughout short fragments of DNA, called DNA barcodes. The same barcode region could be employed for multiple species within a taxonomic group. Ideally the DNA barcode should be variable enough to distinguish closely related species, but also be conserved at intraspecific level. (Pawlowski *et al.*, 2020). DNA barcoding provides a means of identifying known species by sequence similarity with a particular DNA sequence (Bucklin *et al*., 2011).

DNA sequence analysis offers many opportunities for accurate, reliable, rapid, and eventually remote identification of specimens (Bucklin *et al*., 2011). Reasons for barcoding are: works with fragments and for all stages of life; distinguish among species that look alike; reduces ambiguity; enhance public access to biological knowledge, etc.

In contrast, DNA metabarcoding analyses a community of species from environmental (eDNA) or bulk samples (figure 1). Bulk samples contain tissues of many specimens, whereas environmental samples are collected directly from water, sediment, soil or air. Environmental DNA (eDNA) is described as a “pool of genomic material originating from living organisms that remains present in different types of environmental samples” (Pawlowski et al., 2020).

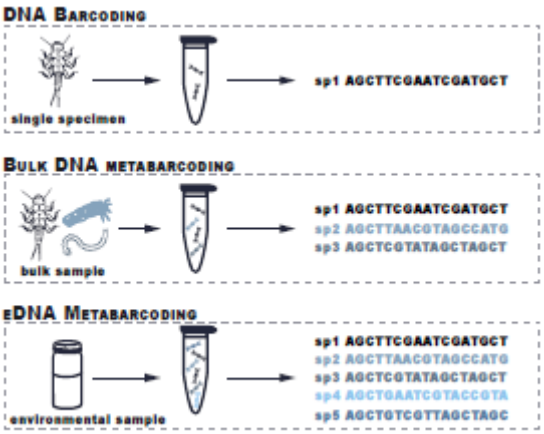

Figure 1 Schematic explanation of barcoding, bulk and eDNA metabarcoding (Pawlowski *et al*., 2020).

The workflow of a typical metabarcoding study includes DNA extraction from bulk or environmental samples. Then, a standardised marker gene is amplified and sequenced (high-throughput sequencing) followed by comparison against reference databases allowing for cost-efficient and reliable community assessments (Elbrecht *et al.*, 2017).

The advent of high throughput sequencing (HTS) technologies brings the opportunity to apply DNA sequencing for environmental biomonitoring programs. In this context, DNA metabarcoding holds great promise for what has been called “biomonitoring 2.0” (Baird and Hajibabaei, 2012). Compared to traditional methods, DNA based techniques increase sensitivity, speed, accuracy and resolution of species identification. Also, it is non-invasive and cost-effective.

First studies on metabarcoding highlighted its capability for taxonomic identification (Gibson *et al.*, 2014; Hajibabaei *et al.*, 2012; Zhou *et al.*, 2013). However, results were inconclusive due to the need of further improvements in: universal primers; DNA preservation and isolation; methods on large scale analysis, etc.

Regarding marine ecosystems, attempts in plankton samples have been performed by Brown et al. (2015), Mohrbeck et al., (2015) and Albaina et al. (2016), among others. While many barcoding studies have allowed accurate marine macroinvertebrates identification (Barroso *et al.*, 2010; Elsasser *et al.*, 2009; Hunt *et al.*, 2010) specifically, Elbrecht *et al*. (2017) have linked this approach to bioassessment and monitoring. The latest, assessed ecological status from both, morphological and DNA barcoding. Problems with current laboratory protocols and reference databases still remained unfixed.

Recent efforts in optimization analytical protocols for fishes enables its identification from fins, fragments, larvae, fillets and eggs. Some studies on fish monitoring have been proved in freshwater (Hänfling *et al.*, 2016; Pont *et al.*, 2018), stating that eDNA metabarcoding can efficiently asses species abundance. In marine environments, most studies are constrained to very small areas (Jeunen *et al.*, 2019; Sigsgaard *et al.*, 2017; Thomsen *et al.*, 2012; Yamamoto *et al.*, 2017) or to family level taxonomic assignments (Thomsen *et al.*, 2016). Additionally, Comparisons between traditional fish monitoring techniques and eDNA methods have also been performed by Kelly *et al* (2017), schmelze and Kinziger (2016), Thomsen *et al*. (2012) or Sigsgaard *et al*. (2017).

Studies on DNA metabarcoding have clearly demonstrated its potential for biological monitoring (Darling *et al.*, 2017; Deiner *et al.*, 2017; Leese *et al.*, 2018) and environmental management (Hering *et al.*, 2018). Additionally, Pawlowski *et al* (2018) goes a step applying metabarcoding to provide DNA biotic indices.

Available data suggest that morphological and metabarcoding based assessment systems are compatible and can even use the very same sampling protocols, but a shift to DNA-based tools comes with both gains and losses (Leese *et al.*, 2018). In addition, additional large-scale studies are needed to validate and improve metabarcoding protocols for routine monitoring (Elbrecht *et al.*, 2017).

Importance of a complete reference database

Metabarcoding aims to assign barcodes to taxa for which reference sequence is available, derived from morphologically identified species. Effectiveness of species identification by DNA metabarcoding depends on a reliable reference database of known taxa. Gaps in reference libraries results in markers that do not overlap with standardized barcodes, generating mislabelled sequences. (Cristescu, 2014; Leese *et al.*, 2018; Mohrbeck *et al.*, 2015; Weigand *et al.*, 2019).

In 2004, the Consortium for the Barcode Life (CBOL; http://barcoding.si.edu) was launched with a mission of developing DNA barcoding as a global standard for the identification of biological species. In partnership with the Census of Marine Life (CoML; http://www.coml.org), CBOL initiated an international campaign for barcoding marine biodiversity (MarBOL; http://www.marinebarcoding.org) (Bucklin *et al*., 2011). Barcoding has also shown success with fishes, leading to the development of Fish Barcode Life (FISH-BOL; http://www.fishbol.org) in 2005.

Nowadays, the Barcode of Life Data System (BOLD (Ratnasingham and Hebert, 2007)) and GeneBank (Benson *et al*., 2013) are the most essential reference libraries for biodiversity monitoring (Weigand *et al*., 2019). However, the gap in the reference library for marine species is relatively large (>70%) with the exception of fish (18%) (Weigand *et al.*, 2019). According to Weigand *et al.* (2019) over 82% of the fishes included in ERMS checklist are barcoded (figure 2), ranging from 100% (71% ≥ 5 barcodes) for the *Holocephali* to 81% (63% ≥ 5 barcodes) for the *Actinopterygii*, with the *Elasmobranchii* coverage is in between (92% ≥ 1 barcodes, 80% ≥ 5 barcodes).

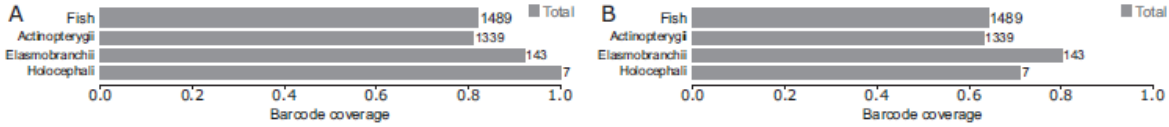

Figure 2. Barcode coverage for marine Fish of the ERMS checklist. (A) Barcode coverage by at least one reference sequence or (B) five reference sequence. Thick bars represent all fish, thin bars represent lower taxonomic rank. Numbers on bars refer to total number of species in checklist. (Weigand *et al*., 2019).

Due to its commercial importance, marine fish is one of the most studied group in terms of DNA barcoding. However, a fair proportion of barcode records remain unknown, especially of non-commercial or not accessible species. Examples on how the lack of reliability of reference databases hampers DNA metabarcoding projects occurred to Elbercht et al., (2017) and to Vasselon et al., (2017). Both studies documented a high proportion of unclassified sequences at species level. These are not isolated cases; in an attempt of assessing fish diversity of the Bay of Biscay (Fraija-Fernández *et al.*, 2020), found that eDNA metabarcoding revealed a high abundance of two species never reported before in those areas.

The incompleteness of DNA barcode libraries is a current bias for DNA based biomonitoring (Elbrecht *et al.*, 2017; Fraija-Fernández *et al.*, 2020; Leese *et al.*, 2018). Hence, this project aims to complete the global reference database to avoiding mislabelled sequences in upcoming studies. For this purpose, the genome of 50 marine fish species was sanger sequenced by PCR amplification of 12s mitochondrial rRNA gene.

Target gene for marine fish DNA barcoding and metabarcoding

Many DNA barcoding campaings for marine fishes had been performed upon the mitochondrial COI gene (Lakra *et al.*, 2011; Landi *et al.*, 2014; Ward *et al.*, 2005), as it is considered the universal metazoan barcode gene (Hebert *et al.*, 2003). The major

advantage of working with the COI gene is that it is maternally inherited, subsequently the taxonomic uncertainty derived from species hybridization is minimized (Ward *et al.*, 2005). Also, it is useful over a broad range of taxonomic level and lacks of overlap between intra- and interspecific variants. This fact is clearly reflected in the large public databases available for COI gene (Bucklin *et al*., 2011).

However, COI gene have been proved to be insufficient for many marine species, especially for those showing Mitochondrial introgression (genus *Thunnus*, for instance). Under these circumstances, concrete regions of the ribosomal RNA gene (internal transcribed spacer) were proposed as more suitable. The ITS evolves rapidly and it has been used for phylogenetic comparisons among closely related taxa (Chow *et al*., 2006). Numerous studies have employed 12S rRNA genes among fish groups (Chow *et al*., 2006; Ardua *et al*., 2010; Cawthorn *et al*., 2012; Ardua *et al*., 2012). In terms of DNA barcoding, consensus on whether is preferably to employ the COI gene or 12S rRNA is not been made up to date, whilst it is recommended the use of both, nuclear and mitochondrial sequences (Ardua *et al*., 2010 and Cawthorn *et al*., 2012). However, for eDNA metabarcoding applications the COI gene is unsuitable (Bylemans *et al.*, 2018).

The target gene for this DNA barcoding project is the 12S rRNA for various reasons. The main one is that this study is part of the biomonitoring programmes carried out by AZTI alongside the Bay of Biscay, in which fish species are identified through the 12S rRNA gene. This is chiefly due to the existence of specific primers for fish species that target the 12S gene, enabling a cost-effective survey. Important to note, is also that the reference database for the 12S gene is more incomplete than the one for COI. As more reliable the reference library, the better the metabarcoding analysis would be.

Metabarcoding studies also require an appropriate marker. Ideally, eDNA metabarcoding primers should amplify a very short fragment (<150 ppb) and be specific to a taxonomic group (Bylemans *et al.*, 2018). The Teleo primer pair (F: ACACCGCCCGTCACTCT; R: CTTCCGGTACACTTACCATG) amplify a short fragment of the 12S rRNA region with high taxonomic coverage and resolution, compared to other primers (Valentini *et al.*, 2016). This marker is the one employed by AZTI for biomonitoring studies, reason for which finding the Teleo region for each fish species is of special concern in this project.

Area of study

This project barcodes Marine fish species from both, Mediterranean Sea and Atlantic Ocean. The First one is a semi-enclosed sea that connects through the strait of Gibraltar to the Atlantic Ocean, and to the Red Sea through Suez Canal. Therefore, Mediterranean

basin is a marine biodiversity hot Spot (Coll *et al.*, 2010). As for its Oceanographic conditions, is important to note that is an oligotrophic basin with high evaporation rates. Sea surface temperature is strongly affected by season. In the Strait of Sicily, a shallow ridge at 400 m depth divides the sea into two main subregions: the western (area = 0.85 million km2) and the eastern (area = 1.65 million km2) (Coll *et al.*, 2010). Biological production is higher at western area, whereas the eastern shows higher temperature and salinity.

The Mediterranean basin hosts endemic species such as *Posidonia oceanica*, vermetid reefs and coralligenous assemblages, that coexist with biota derived from the Atlantic Ocean. Also, Is the spawling grounds of the eastern Atlantic bluefin Tuna (*Thunnus thynnus*) (Coll *et al.*, 2010). The large Size of the Mediterranean deep-sea ecosystem includes areas with depths greater than 3000m. This unusual feature, classified as deep sea, shows bathyal and abyssal taxonomic groups which had not been studied yet.

Regarding Atlantic Ocean, sampling area includes Portugal, Cadiz and Galicia. In particular, Marine ichthyofauna of Portugal is characterises by richness of species from different adjacent sources. Portugal´s geographic location serves as meeting area for fish species from the Mediterranean Sea, Subtropical Northeastern Atlantic and the depths of the mid-Atlantic ridge (Costa *et al.*, 2012). Hydrodynamics of this area is characterized by coastal upwellings during summer. Of special concern is the Gulf of Cadiz Slope Current, which inflows Atlantic waters into the Mediterranean basin (Peliz *et al*., 2009). Major fish species in these landings are*: Sardina pichardus*, *Engraulis encrasicolus, Trachus* spp., *Micromesistius potassou*, thunnus thynnus, etc. (Coll *et al.*, 2014).

Located off the north-west coast of Spain, the Galician continental shelf (<200 m depth) and upper slope (200-500 m) also holds a great promise to pelagic and demersal fisheries as *Sardina pilchardus*, *Merluccius merlucccis* or *Micromesistus poutassou*, for instance (Fariñ *et al*., 1997). These fish species are driven along the coast due to the great abundance of plankton biomass supported by upwelling processes during summer. The general circulation pattern of Galicia is characterized by the convergence between the anticyclonic gyre of the Bay of Biscay and the inflowing current of Portugal (Tenore *et al.*, 1995).

To summarize all that has previously been said, this study aims to increase the reference DNA barcode database, filling the gaps of Azti´s eDNA metabarcoding programmes. The target gene was the 12S rRNA, as is it specific to marine fish, and includes the Teleo

region, which is the marker employed for metabarcoding. The further goal is to correctly apply metabarcoding to environmental monitoring and fish detection.

OBJECTIVES

The main objective is to complete the global reference database for improving taxonomic identification of environmental DNA. Secondary objectives include:

- To extract DNA of species for which no reference sequence is available in public databases
- To amplify the 12S mitochondrial rRNA gene from the extracted DNA and sanger sequence it.
- To verify and cure each sequence, submitting the obtained sequences to GeneBank.
- To obtain the Teleo region for each final sequence.
- To study the interspecific and intraspecific divergence.

MATERIALS AND METHODS

Description of samples

This project utilises samples collected by an oceanographic campaign (SUMMER), which took place in 2020. SUMMER (Sustainable Management of Mesopelagic Resources) covered ecological aspects of mesopelagic communities.

The survey had two main study sites (figure 3) in eastern Iberia- western Mediterranean Sea (oligotrophic region), and two more in western Iberia (productive region). In each of the 4 sites repeated samples were performed day and night during 3 journeys. (Olivar, P. and UTM-CSIC, 2020).

Figure 3. Pathway followed by Sarmiento de Gamboa Oceanographic vessel during SUMMER 2020. Arrows represent areas were CTD was thrown, while triangles are sampling sites of pelagic and mesopelagic species (Olivar, P. and UTM-CSIC, 2020).

According to figure 3, samples of pelagic and mesopelagic species were taken by trawling at Baleares, Alboran, Cadiz and Lisbon. Samples were preserved in ethanol at -20ºC. These include biological tissues from pelagic and mesopelagic fish species (table 2).

Table 2. Species for DNA barcoding.

Species	Number of samples	Species	Number of samples
Argyropelecus hemigymnus	23	*Cyclothone pseudopallida*	3
Zeus faber	2	*Ceratoscopelus warmingii*	10
Microcochirus variegatus	2	*Diaphus holti*	14
Spondyliosoma cantharus	2	*Hygophum benoiti T*	14
Umbrina canariensis	2	*Hygophum reinhardtii*	4
Umbrina cirrosa	2	*Lampanyctus alatus*	5
Chelidonichthys lucernus	1	*Lampanyctus crocodilus*	4
Conger conger	1	*Lobianchia dofleini*	18
Argyrosomus regius	2	*Lepidophanes gaussi*	5
sarda sarda	2	*Lampanyctus pusillus*	16
Molva molva	2	*Maurolicus muelleri*	9
Argyropelecus sladeni T	5	*Myctophum punctatum*	10
Benthosema glaciale	14	*Notolychnus valdiviae*	7
Benthosema suborbitale	6	*Photostomias guernei*	5
Cyclothone alba	3	*Stomias _*	5
Cyclothone braueri	20	*Sigmops elongatus*	8
Chauliodus danae	10	*Vinciguerria attenuata*	4
Chauliodus sloani	15	*Vinciguerria nimbaria*	20
Ceratoscopelus maderensis	13	*Valenciennellus tripunctulatus*	10
Cyclothone pallida	2	*Dicologlossa cuneatea*	5
Lepidorhobus boscii	5	*Lepidorhombus whiffiagonis*	5
Mullus surmuletus	5		

DNA extraction

As seen in table 2, each fish species had more than one sample. For this project just four samples of each specimen, if possible, were processed. DNA was extracted from biological tissues using the Wizard R Genomic DNA Purification kit (Promega, WI, USA) in a 100 µl of Milli-Q water final elution. The protocol was slightly modified: for each sample to be extracted was added: 250 µl Nuclei Lysis Solution; 60 µl of a 0,5M EDTA solution (pH 8); 20 µl of 20mg Proteinase K; and 3 µl of RNAasa solution. The mixture was incubated overnight at 56ºC. Once the extraction was performed, 100 µl of Protein

precipitation (Promega, WI, USA) solution was added to each sample. Later each of them was vortexed at high speed, chilled on ice for 5 minutes and centrifugated for 4 minutes at 13 xg. The supernatant, containing DNA was transferred to a clean 1,5 ml centrifugate tube containing 300 µl of room temperature isopropanol. After mixing and leaving the mixtures at room temperature for 5 minutes they were once again centrifugated, under the same conditions, this time leaving the supernatant behind. Each pellet sample (DNA sample) was washed with 300 µl of room temperature ethanol. After a third and final centrifugation the supernatant was decanted, leaving each sample pellet to air-dry for 15 minutes in an inverted tube. A final rehydration on 100 µl of Mili-Q water prepared the final DNA extracted samples.

Genomic DNA integrity was assessed by electrophoresis, migrating DNA on a 1.0% agarose TAE buffer gel with a Qubit R 2.0 Fluorometer (Life Technologies). DNA purity was assessed using the Nanodrop ND-1000 (Thermo Scientific) system. 5 µl of each extracted DNA sample was eluted with the aim of performing DNA barcoding of single species. The images of the gels for both, DNA extraction and amplification were taken with a Chemi Doc XRS molecular imager (Bio Rad).

PCR Amplification and Sanger sequencing

Target gene was 12 mitochondrial rRNA, as it has been proved suitable for marine fishes. PCR amplification was performed using universal primers proposed by Jin *et al*. (2013) (table 3)

Table 3. Primers used in this study (Jin *et al*.,2013).

Primer name	Sequence
Marinefish-12SrRNA-F	ACTAAAGCATAACACTGAAGAT
Marinefish-12SrRNA-R	TTCATTTCTCTTTCAGCTTTCC

Each individual eluted DNA sample was amplified in a total volume of 15 µl using 7,5 µl of Phusion High-Fidelity PCR Master Mix (Thermo Scientific); 1 µl of each primer (forward and reverse); 1 µl of 1mg/l BSA (Thermo Scientific); and 4,5 µl of genomic DNA. The conditions of the PCR were as follows: pre-denaturation at 95°C for 5 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 75 s; and final extension at 72°C for 5 min (Jin *et al*., 2013). PCR products were electrophoresed on a 1.7% agarose TAE buffer gel.

PCR products were purified with 4 µl of ExoSAP-IT (Affymetrix) and Sanger sequenced, both forward and reverse.

Sequence analysis

Sequences obtained from individual PCR amplifications were edited and trimmed, to remove quality bases below 30, using SeqTrace 0.9.0 (Stucky, 2012). In order to obtaining the final sequences, the analysis was performed bidirectionally Forward and reverse trace files were visualized with SeqTrace, whenever a base differed from forward and reverse, the option with the higher quality score was elected. The analysis can be performed this way because the quality of the forward sequencing is higher at the beginning, whereas at the end, the reverse sequencing is more robust. As an example, figure 4 shows the traces files analysis for *Vinciguerria attenuata.* The upper trace file corresponds to the forward sequence, the one at the bottom is the reverse sequence and the numbers above each peak represent the quality score. The yellow bar highlights a mismatch between forward and reverse. Below the trace files is located the final sequence to be edited, where can be seen that the A base was elected over the C because of the higher quality score of the reverse sequence.

Figure 4. Trace files analysis of forward (upper) and reverse (lower) sequences for *Vinciguerria attenuata* using Sec-trace. Numbers represent the sequencing quality.

This discordance occurred at the end of the sequence. However, as seen in figure 5, midway through the sequencing the quality is high for both, reverse and forward sequences.

Figure 5. Trace files analysis of forward (upper) and reverse (lower) sequences for *Myctophum punctatum* using Sec-trace. Numbers represent the sequencing quality. There are not mismatching bases.

An alignment of the obtained sequences with TELEO primer pair, using ClustalW (Thompson *et al.*, 1997) in Bioedit (Hall, 1999) was eventually performed to finding the TELEO region.

To observe the genetic variability among individuals from the same fish species, sequences were aligned followed by a plot identities analysis through Bioedit (Hall, 1999). The aligned sequences were subjected to a phylogenetic analysis using Maximum Likelihood (ML) in bioedit (Hall, 1999). Also, identity matrices were calculated at spicies and genus level.

RESULTS AND DISCUSSION

DNA extraction and PCR amplification

Out of the 141 DNA extraction performed 30 showed no result when migrating the DNA on Agarose gel and were subsequently repeated. Furthermore, many of the DNA samples were degraded; as shown in figures 6-9 DNA was considered of high quality when a clear bar appeared at the height of Qubit fluorometer.

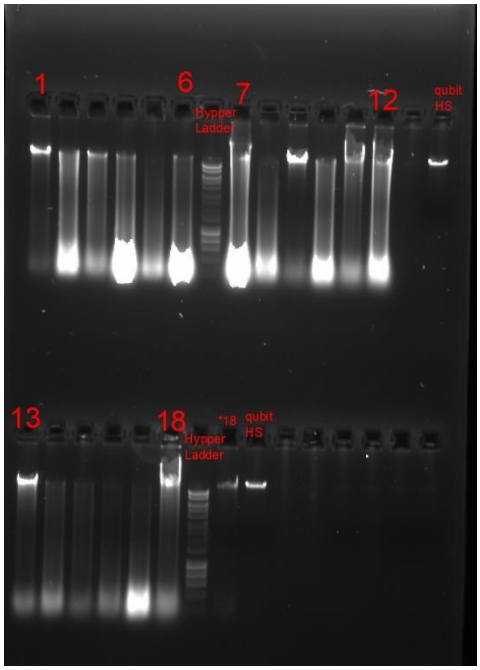

Figure 6. DNA integrity assessment on a DNA on a 1.0% agarose TAE buffer gel, samples from 1 to 18.

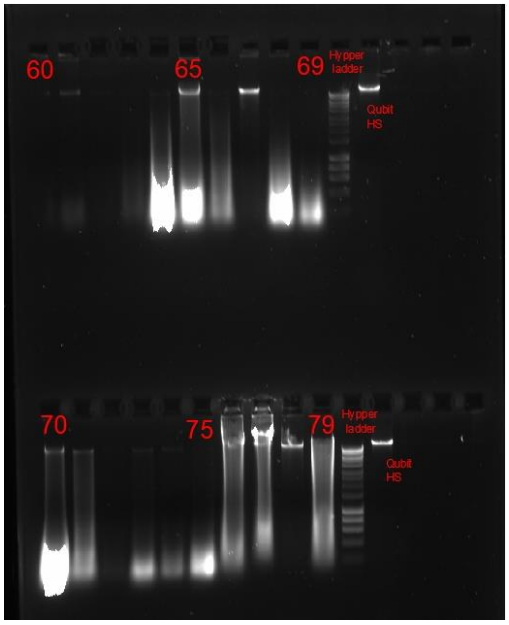

Figure 7. DNA integrity assessment on a DNA on a 1.0% agarose TAE buffer gel, samples from 60 to 79.

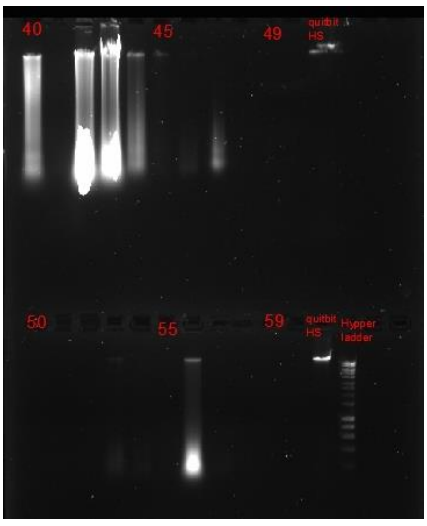

Figure 8. DNA integrity assessment on a DNA on a 1.0% agarose TAE buffer gel, samples from 40 to 59.

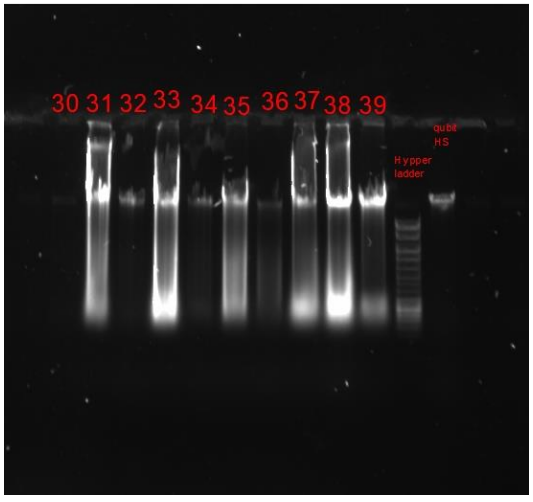

Figure 9. DNA integrity assessment on a DNA on a 1.0% agarose TAE buffer gel, samples from 30 to 39.

The quality of samples was very diverse. For instance, samples 1, 8, 13, 67, 65 or 77 are considered of high quality, whereas samples from 2 to 7; from 14 to 18; and from 30 to 39 exhibit very degraded DNA. Other samples as 46 to 55 showed no DNA. Errors during laboratory procedures could have made some extractions fail, therefore is expected that these samples do not amplify by PCR. On the other hand, DNA degradation occurs when the preservation of samples is not properly performed, or when the sampling method is too aggressive.

PCR products were considered positive when a single band of expected size was visualized (figures 10-13).

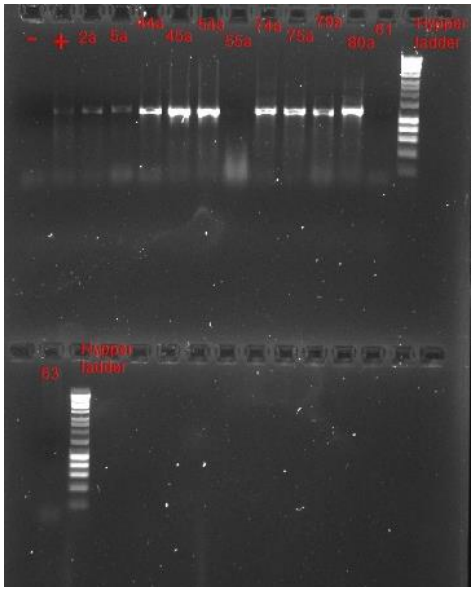

Figure 10. 12S PCR products, repeated samples.

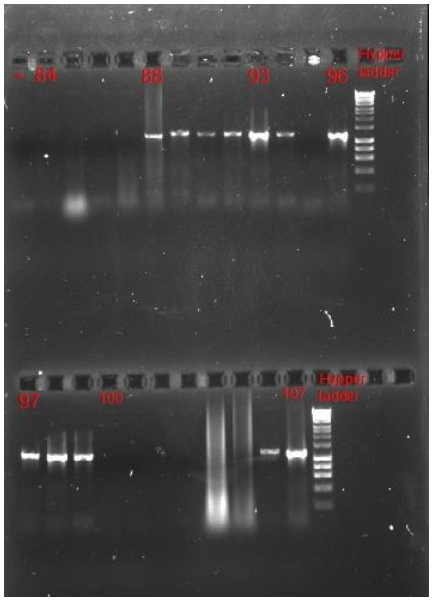

Figure 11. 12S PCR products, samples from 84 to 107.

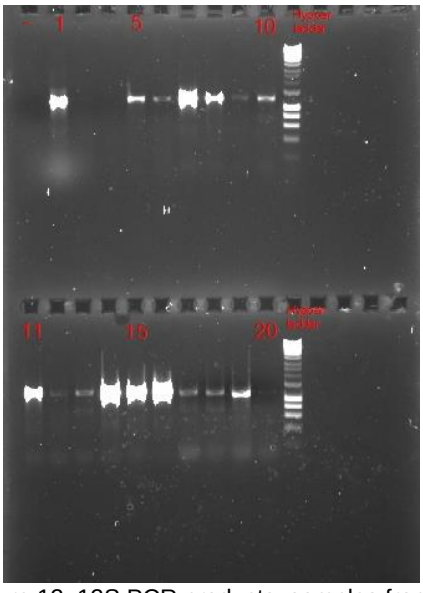

Figure 12. 12S PCR products, samples from 1 to 20.

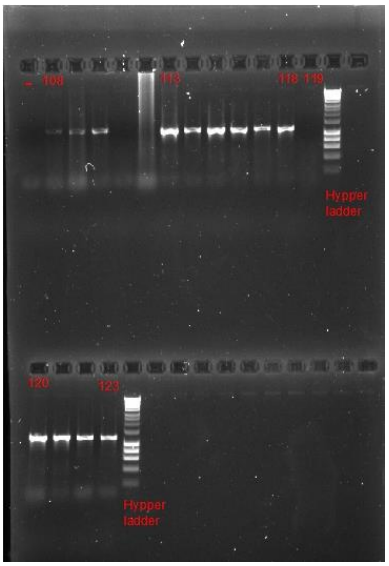

Figure 13. 12S PCR products, samples from 108 to 123.

PCRs that did not work were repeated by shifting the concentration of DNA. Despite these attempts, out of 141 samples 45 never amplified, which might be due to the quality of samples. As seen in figures 6 to 9, the quality of most of the samples was poor and some even did not show any DNA when migrating the agarose gel. For the latest, it was expected that the amplification was not going to occur. Besides, regarding the rest of the samples that showed DNA but not PCR product two hypotheses had been stated: the DNA might be so degraded that the amplification gets inhibited, or the primers employed are not suitable for selected species.

Table 4 summarizes the DNA extraction and 12S PCR amplification results for each sample. there is more than one sample for every fish species, therefore, even if one procedure fails, the result for one specimen is supported by the remaining samples.

Table 4. Scientific name, DNA extracted concentration and PCR results for each sample. In grey are the samples for which no PCR worked.

Sample ID	**Fishbase Scientific Name**	**extracted DNA concentration (ng/µL)**	**PCR**
1	*Zeus faber*	50,08	yes
2	*Microchirus variegatus*	313,53	yes
3	*Spondyliosoma cantharus*	194,9	yes
5	*Chelidonichthys obscurus*	166,39	yes
6	*Umbrina canariensis*	387,04	yes
7	*Umbrina cirrosa*	347,05	yes
8	*Chelidonichthys lucernus*	463,55	yes
9	*Conger conger*	683,05	yes
10	*Zeus faber*	94,13	yes
11	*Chelidonichthys lucernus*	393,01	yes
12	*Conger conger*	473,77	yes
13	*Spondyliosoma cantharus*	149,68	yes
14	*Umbrina canariensis*	75,37	yes
15	*Umbrina cirrosa*	64,17	yes
16	*Argyrosomus regius*	87,5	yes
17	*Argyrosomus regius*	179,34	yes
18	*Scorpaena porcus*	395,16	yes
19	*sarda sarda*	32,89	yes
20	*sarda sarda*	409,52	yes
21	*Caranx hippos*	1305,15	yes
22	*Caranx hippos*	922,71	yes
23	*Chelidonichthys cuculus*	139,74	yes
24	*Chelidonichthys cuculus*	438,41	yes
25	*Trigloporus lastoviza*	1896,95	no
26	*Trigloporus lastoviza*	16,54	yes
27	*Diplodus vulgaris*	1,12	yes

28	*Diplodus vulgaris*	1443,56	yes
29	*Phycis blennoides*	61,66	yes
30	*Phycis blennoides*	1,04	yes
31	*Scorpaena porcus*	64,08	yes
32	*Scorpaena scrofa*	19,15	no
33	*Molva molva*	897,73	no
34	*Molva molva*	3,27	yes
35	*Scorpaena scrofa*	813,63	no
36	*Labrus bimaculatus*	4,82	yes
37	*Labrus bimaculatus*	1398,07	yes
38	*Molva macrophthalma*	1163,65	no
39	*Molva macrophthalma*	1810,79	no
40	*Argyropelecus hemigymnus*	216,03	no
41	*Argyropelecus hemigymnus*	3,01	yes
42	*Argyropelecus hemigymnus*	1656,31	no
43	*Argyropelecus hemigymnus*	2043,02	no
44	*Benthosema glaciale*	76,95	yes
45	*Benthosema glaciale*	1,51	yes
46	*Benthosema glaciale*	7,22	yes
47	*Benthosema glaciale*	40,94	yes
48	*Cyclothone alba*	7,71	yes
49	*Cyclothone alba*	-0,03	no
50	*Cyclothone alba*	0,41	no
51	*Cyclothone braueri*	-1,7	yes
52	*Cyclothone braueri*	107,82	no
53	*Cyclothone braueri*	16,44	no
54	*Cyclothone braueri*	2,62	no
55	*Cyclothone pallida*	0,84	yes
56	*Cyclothone pallida*	4,06	no
57	*Cyclothone pseudopallida*	214,56	yes
58	*Cyclothone pseudopallida*	60,32	yes
59	*Cyclothone pseudopallida*	26,58	yes
60	*Chauliodus danae*	16,51	yes
61	*Chauliodus danae*	89,34	no
62	*Chauliodus danae*	8,92	no
63	*Chauliodus danae*	30,77	no
64	*Chauliodus sloani*	352,18	no
65	*Chauliodus sloani*	344,78	no
66	*Chauliodus sloani*	96,66	no
67	*Chauliodus sloani*	21	no
68	*Ceratoscopelus maderensis*	533,42	no
69	*Ceratoscopelus maderensis*	85,83	yes
70	*Ceratoscopelus maderensis*	893,12	yes
71	*Ceratoscopelus maderensis*	119,6	yes
72	*Ceratoscopelus warmingii*	5,4	yes

73	*Ceratoscopelus warmingii*	101,6	yes
74	*Ceratoscopelus warmingii*	64,8	yes
75	*Ceratoscopelus warmingii*	269,32	no
76	*Diaphus holti*	905,43	yes
77	*Diaphus holti*	1345,55	yes
78	*Diaphus holti*	15,07	yes
79	*Diaphus holti*	396,46	no
80	*Hygophum benoiti T*	881,79	yes
81	*Hygophum benoiti T*	19,2	yes
82	*Hygophum benoiti T*	9,97	no
83	*Hygophum benoiti T*	27,42	no
84	*Hygophum reinhardtii*	488,65	no
85	*Hygophum reinhardtii*	102,92	no
86	*Hygophum reinhardtii*	180,95	no
87	*Hygophum reinhardtii*	31,95	no
88	*Lobianchia dofleini*	1,25	yes
89	*Lobianchia dofleini*	1643,15	yes
90	*Lobianchia dofleini*	1602,24	yes
91	*Lobianchia dofleini*	659,76	yes
92	*Lampanyctus crocodilus*	318,29	no
93	*Lampanyctus crocodilus*	4,84	yes
94	*Lampanyctus crocodilus*	8,97	yes
95	*Lampanyctus crocodilus*	4,87	yes
96	*Lampanyctus alatus*	11,12	no
97	*Lampanyctus alatus*	1,19	yes
98	*Lampanyctus alatus*	1,05	yes
99	*Lampanyctus alatus*	273,94	yes
100	*Lepidophanes gaussi*	10,16	no
101	*Lepidophanes gaussi*	4,4	no
102	*Lepidophanes gaussi*	11,37	no
103	*Lepidophanes gaussi*	3,67	no
104	*Lampanyctus pusillus*	3,35	yes
105	*Lampanyctus pusillus*	2,68	yes
106	*Lampanyctus pusillus*	1674,02	yes
107	*Lampanyctus pusillus*	23,22	yes
108	*Maurolicus muelleri*	59,41	yes
109	*Maurolicus muelleri*	34,45	yes
110	*Maurolicus muelleri*	78,09	yes
111	*Maurolicus muelleri*	2,59	no
112	*Myctophum punctatum*	15,05	yes
113	*Myctophum punctatum*	379,8	yes
114	*Myctophum punctatum*	560,48	yes
115	*Myctophum punctatum*	56,37	yes
116	*Notolychnus valdiviae*	8,31	yes
117	*Notolychnus valdiviae*	36,72	yes

118	*Notolychnus valdiviae*	149,95	yes
119	*Notolychnus valdiviae*	9,78	yes
120	*Photostomias guernei*	12,43	yes
121	*Photostomias guernei*	18,02	yes
122	*Photostomias guernei*	29,59	yes
123	*Photostomias guernei*	259,44	yes
124	*Vinciguerria attenuata*	100,84	no
125	*Vinciguerria attenuata*	7,52	yes
126	*Vinciguerria attenuata*	104,1	yes
127	*Vinciguerria attenuata*	307,21	yes
128	*Vinciguerria nimbaria*	16,82	yes
129	*Vinciguerria nimbaria*	4,34	yes
130	*Vinciguerria nimbaria*	1,31	yes
131	*Vinciguerria nimbaria*	2,08	yes
132	*Valenciennellus tripunctulatus*	10,5	no
133	*Valenciennellus tripunctulatus*	58,5	no
134	*Valenciennellus tripunctulatus*	2,4	no
135	*Valenciennellus tripunctulatus*	80,79	no
136	*Stomias _*	3,16	yes
137	*Stomias _*	3,04	yes
138	*Sigmops elongatus*	2,19	yes
139	*Sigmops elongatus*	1,88	yes
140	*Sigmops elongatus*	-2,57	yes
141	*Sigmops elongatus*	-0,65	yes

According to table 4, is important to noticing that although 12S amplification did no worked for *Hygophum reinhardtii,* it did for its relative *Hygophum benoiti T.* Besides, the 12s region did not amplify for any species of *Chauliodus* genre and showed troubles for the genus *Cyclothone.* having 12 samples of the latest, the PCR amplification was only proficient for 6.

Of special concern is to mention that 12S PCR amplification completely failed for the following fish species: *Scorpaena scrofa, Valenciennellus tripunctulatus, Lepidophanes gaussi, Hygophum reinhardtii, Chauliodus danae, Chauliodus sloani* and *Molva macrophthalma*. The whole workflow of extraction and PCR amplification was repeated for these fish species, utilizing other specimens if possible, in order to dismiss laboratory errors. If needed, changes in DNA elution were also made. However, 12S amplification never worked, which might suggest that Marinefish 12S primers do not amplify for these fish species. To verify this hypothesis future attempts employing different primers need

to be performed. Having this been said, the possibility of executing laboratory mistakes is not being dismissed. Moreover, the fact that *Scorpaena scrofa* did not amplify, exhibit inaccuracies during analytical procedures; as this fish species has already been sequenced (GenBank: MT903912.1).

Sequence verification and TELEO region identification

96 samples were Sanger sequenced with both, forward and reverse primers, which makes a total of 192 sequences. Out of them, only 123 were successfully sequenced. Target gene (12S rRNA) is about 1000 ppb length, however from the 123 sequencies only 76 were more than 702 ppb length (figure 13). As seen in figure 14, most of the sequences were between 900 and 1000 ppb length; besides, 21 sequences had between 300 and 500 ppb and 8 of them not even reached 300 ppb.

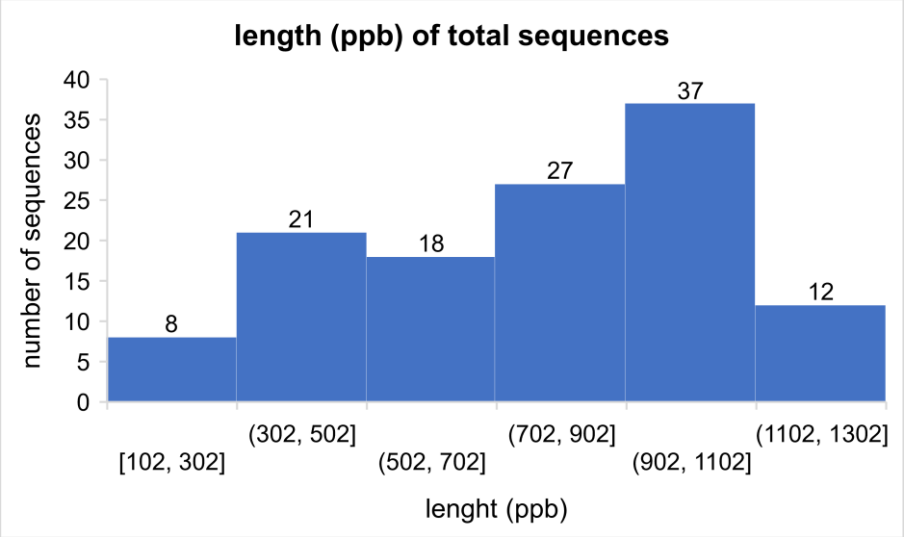

Figure 14. Length in ppb of the total sequences (123), including forward and reverse. X axis show the length in ppbs, whereas de Y axis represents the number of sequences. The numbers over the bars the numbers over the bars denote the number of sequences specific for each interval.

As the Teleo region is approximately located between 800 and 900 ppb, the alignment of the sequences that were too short could not be performed. Additional troubles occurred when aligning forward and reverse sequences if the length of both did not match. A well curated final sequence could only be obtained if the forward and reverse sequenced reached, at least 800 ppb and the quality score was over 30. Eventually, the fish species for which a successful sequence was obtained are the following: *Argyrosomus regius, Benthosema glaciale, Ceratoscopelus maderiensis* (2 individuals), *Ceratoscopelus warmingii* (3 individuals), *Chelidonichthys cuculus* (2 individuals), *Chelidonichthys lucerns, Diaphus holti* (2 individuals), *Labrus bimaculatus* (2 individuals), *lampanyctus alatus* (2 individuals), *Lampanyctus pusillus* (4 individuals),

Lepidorombus whiffiagonis (3 individuals), *Lobianchia dofleini* (2 individuals), *Microchirus variegatus*, *mullus surmuletus* (3 individuals), *Myctophum punctatum* (3 individuals), *Notolychnus valdiviae* (3 individuals), *Photostomias guernei* (3 individuals), *Sarda sarda , Stomias sp* (2 individuals), *Umbria canariensis* (2 individuals), *Umbria cirrosa, Viciguerria attenuata* (2 individuals), *Viciguerria nimbaria* (3 individuals) and *Zeus faber*. As an example, some of the sequences in FASTA format are shown in figures 15 and 16, the rest are found in Annex I.

```
>..\Sequences\FOR.ab1\.ab1\B3+16+For.ab1, ..\Sequences\REV.ab1\.ab1\B3+16+Rev.ab
AAAAGCTTGGGTCCTGACTTTACTATCAACTTTAGCTATATTTACACATGCAAGTATCCGCACCCCTGTGAGAATGCCCT
AATAGCTCCCTGCCCGGGAACAAGGAGCTGGTATCAGGCACAACTAACTGTAGCCCACGACACCTTGCTTTGCCACACCC
TCAAGGGAACTCAGCAGTGATAGACATTAAGCCATAAGTGAAAACTTGACTTAGTTAAAGCTAAGAGGGCCGGTTAAACT
CGTGCCAGCCACCGCGGTTATACGAGAGGCCCAAGTCGATAGTCAACGGCGTAAAGAGTGGTTAGAAGGAGCCCATTACT
AAAGCCGAACACCCTCAAAGCTGTTATACGCACCCGAAGGTGAGAAGCCCATCCACGAAAGTGGCTTTACAACCTTGAAT
CCACGAAAGCTATGATACAAACTGGGATTAGATACCCCACTATGCTTAGCCCTAAACATTGACAACAACATACACCTGTT
GTCCGCCTGGGAACTACGAGCATCAGCTTGAAACCCAAAGGACTTGGCGGTGCTTTAGATCCACCTAGAGGAGCCTGTTC
TAGAACCGATAACCCCCGTTCAACCTCACCCTTCTTTGTTTCCCCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGA
AGGACTTATAGTAAGCAAAATTGGTACAACCCTAAACGCCAGGTCGAGGTGTAGCGTATGGAAGGGGAAGAAATGGGCTA
CATTCTCTAACACAGAGAAAACGAATGATGTACTGAAATACACGTCTGAAGGAGGATTTAGCAGTAAGCAGGAAATAGAG
TGTCCCGCTGAAATTGGCCCTGAAGCGCGCACACACCGCCCGTCACTCTCCCCAAACTAATTGAATTCAATTAAATAAAA
CCCCACCACAGTAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAAAAATCAGGGCATAGCT
AAGACAGAAAAGCATCTCCCTTACACTGAGCAGTCATCCGTGCAAATCGGATTGCCCTGACGCCCATTAGCTAGCCGCCT
CCACTAAAAACAACAAGTCCCCATCAATAACCCCTAAGACACTCAAGACAACACTAAACAAACCATTTTCCTCCCAAGTA
CGGGCGACAGAAAAGGA
```

Figure 15. 12S sequence of *Argyrosomus regius.*

```
>..\Sequences\FOR.ab1\.ab1\C10+44+For.ab1, ..\Sequences\REV.ab1\.ab1\C10+44+Rev.
ACTTTACTGTCCGCCCCCGCCAAGTTTATACATGCAAGTATCCGCACCCCAGTGAGAATGCCCCTAAACCCCCACCCGGA
AATGAGGAGCAGGCATCAGGCACACCCCTTCGTAGCCCATGACGCCTTGCACAGCCACGCCCCCACGGGAACTCAGCAGT
GATAAATTTTAGGCAATAAGTGCAACCTTGACCTATCTATGGCTAAGAGGGCCGGTAAATCTCGTGCCAGCCACCGCGGT
CATACGAGTGGCCCAAGTGGACAGCCAACGGCGTAAAGCGTGGTTAAGGACCCCCCCCCGATTAAAGCAGAACCCCCTCA
AGGCCGTCATACGCATCCGACAGGGTGAAACCCCACCTCGAAAGTGACTTTAACTCACCTGACCCCACGAAAACTGAGAA
ACAAACTGGGATTAGATACCCCACTATGCTCAGCCGTAAACACTGATAGAAACCCTACGACACCTATCCGCCTGGGGACT
ACAAGCACTAGCTTCAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTGTCCTAGAACCGATAACCC
CCGTTCAACCTCACCACTTCTTGCCTAATCCGTCTATATACCTCCGTCGTCAGCTTACCCTGTGAAGGACTCATAGTAAG
CTTAACTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCATACGAAGTGTGCAAGAGATAGGCTACATTTTCTGATTCA
GAATACTACGAAAGAGGAAATGAAAAGCCCTCCGAAGGCGGATTTAGCAGTAAGCAAGGAATAGAGCGCCCCGCTGAAAC
TGGCCCTGAAGCGCGTACACACCGCCCGTCACTCTCCCCAAACCCAACCAACCCCTCACTAACATATATCACCCCCTGGA
TAAAGGGGAGGCAAGTCGTAACATGGTAGGAGTACCGGAAGGTGCCCCTGGCTAACAGGGCGTGGCTAAACAGCAAAGCA
CCTCCCTTACACCGAGAAGATGTCCGTGCAACTCGGACCGCCCTGAACCTAAAAAGCTAGCCCAACCCCTACAAAAACAG
CCCCCTATAAACCACTCCCTACAAGCTAATCAACAAAACATTTTACCTCCCCAGTA
```

Figure 16. 12S sequence of *Benthosema glaciale.*

These results constitute a step ahead for increasing the reference database currently employed in marine biomonitoring. Intending to primarily solve one of the main obstacles for AZTI´s monitoring programmes, this DNA barcoding project indeed is an advent to global knowledge. Weigand *et al* (2019) stated that the reference library gaps of marine groups should be filled to maximize taxonomic assignment in metabarcoding reads. He also noted the importance of performing this work at the species-level. As important as the completeness of reference data, is the fact that libraries are supplied with reliable barcode references. The results proposed in this study meet with the compulsory requirements for DNA barcodes, which are: a) It has to derive from an accepted gene region, b) It has to demonstrate at least 75% of contiguous high-quality bases or <1% Ns, c) It has to be associated with forward and reverse trace files and d) It has to be related with a voucher specimen (Weigand *et al.*, 2019).

Pretending to fill the gaps of barcoding libraries several countries including Australia (Ward *et al*., 2005); Canada (Hubert *et al*., 2008); Antarctic Scotia (Rock *et al.*, 2008); Alaska and Pacific Arctic (Mecklenburg et al. 2011); Amazon (Ardura et al. 2010); North America (Aprila *et al.*, 2011); India (Lakra *et al*., 2011); Eastern Nigeria (Ude, 2020) and Japan (Zhang and Hanner 2011) have had barcoded marine and freshwater fishes (Nwani *et al.*, 2011). Nevertheless, all of them employed the COI region as target gene. It is undoubtedly that COI gene performs well in terms of amplifying fish species, however, failures in species discrimination had also been reported (Rock *et al.*, 2008). The main problem occurs when applying eDNA metabarcoding to fish detection. Because COI gene is universal for metazoans, not only fish species are amplified, but also the rest of the organisms present in the water column. So, despite the large reference database for COI, many COI metabarcoding studies provide low-resolution taxonomic information (Deagle *et al.*, 2014). For that reason, specific markers to fish detection, such as 12S or 16S rRNA genes may offer a solution. Still, gaps in reference libraries for 12S marine and freshwater fish is very low compared to COI (Weigand *et al.*, 2019).

The originality of this work resides in the fact these fish species have never been sequenced before (at least using 12S rRNA gene). Thus, it represents a tool for the conservation of diversity. It is also the first time that the Teleo region is identified for this fish species. Results are shown in table 5.

Table 5. Teleo region for each fish species.

Fish species	Teleo region
Ceratoscopelus maderiensis	CCCCAAGCCCAGCATATATTTACCATATGAAGCCAAATGGGCAAAGGGGAGGCAAGTCGTAA
Ceratoscopelus warmingii	CACTCTCCCCAAGCCCAACATATATACATCATATGAAGCCAAATGGGCAAAGGGGAGGCAAGTCGTA
Chelidonichthys obscurus	TCCCCAAACTAATTAAATTCAATTAAATAAAACCCCATCACAGTGAAGGGGAGGCAAGTCGTAAC
Micochirus variegatus	CTCCCGTATATAACACATTAAAACCTATTTATAATACCACACCACAATTATTAGGAGAGGAAAGTCGTAAC
Myctophum punctatum	CCCCGAAGTCTCTCTACAGCCTTAACTTATAGCACAAAACTGACAAAGAGGAGGAAAGTCGTAAC
Notolychnus valdiviae	CCCCACAACTTAACCTTAGTACAACATAATGAACCAACAAGTATTCAGGGGAGGCAAGTCGTAAC
Photostomias guerni	CCCCGCGTTAATCGTTTTGTCTAATTAAGAAGTAAACCAAACAAAGGGGAGGCAAGTCGTAAC
Lepidorhombus whiffiagonis	CCCCGAGCTACGAAGTACACATAACTAAAACCCTATAACTGCAAAGGGGAGGAAAGTCGTAA
Mullus surmuletus	CCCCAAGCTCCTGGACCCTAATCCTACTTAAACCCTAACAAATGCGAAGGAGAGGAAAGTCGTAA
Labrus Bimaculatus	CCCCGAAACTATGTATCTTAATACTTAATACCTTAAAACTCCAAAGGGGAGGCAAGTCGTAA
Diaphus holti	CCCCGAAACTATGTATCTTAATACTTAATACCTTAAAACTCCAAAGGGGAGGCAAGTCGTAA
Chelidonichthys cuculus	CCCCAAGCTCACAAATTAATTAATTAAAACCCTAAGGTCGCAAAGGGGAGGCAAGTCGTAA
Lampanyctus alatus	CCCCAATTCCTACCCTGTCATAACCTATAGTCTTAAATGGATAAAGGGGAGGCAAGTCGTAA
Lobianchia dofleini	CCCCGAACCTACTCGCCAAGTAACATAACACGCCAACCGGACAAAGAGGAGGCAAGTCGTAA
Vinciguerria nimbaria	CCCCTACTCACCCCTCTTAGTACATAAAACCCTATTTCTAATAAGGGGAGGCAAGTCGTAA
Umbrina cirrosa	CCCCAAACTAATTGAGTTCAATTAAATAAAACCCCATCACAGTAAAGGGGAGGCAAGTCGTAA
Umbrina canariensis	CCCCAAACTAATTAAATTCAATTAAATAAAACCCCATCACAGTGAAGGGGAGGCAAGTCGTAA

The Teleo primer pair is the marker choice for AZTI´s metabarcoding programmes as it is specific to fish species. The importance of selecting an appropriate and specific marker for metabarcoding studies has already been described (Deagle *et al.*, 2014; Valentini *et al.*, 2016). The accuracy of a metabarcoding study depends on the selectivity of the elected marker. Mitochondrial rRNA genes provide an optimal taxonomic resolution allowing the design of more conserved primers (Deagle *et al.*, 2014).Recent studies have demonstrated that Teleo primers are able to recover highly degraded eDNA and thus, detect high proportion of fish taxa (Bylemans *et al.*, 2018). Teleo primers amplify a short fragment of 12S rRNA gene, which is our target gene; is important assuring that every analysed 12S sequence reaches the Teleo region. Figure 17 is the result of the aligned sequences with Teleo primers pair. The numbers above represent the length of the sequence and the first two rows correspond to forward and reverse primers sequences. Below the primer set, are settled the analysed sequences. The alignment was performed following ClustalW (Thompson *et al.*, 1997). The region between both primers is the Teleo region. It can be seen how one *Vicinguerria nimbaria* specimen did not come to the desired length.

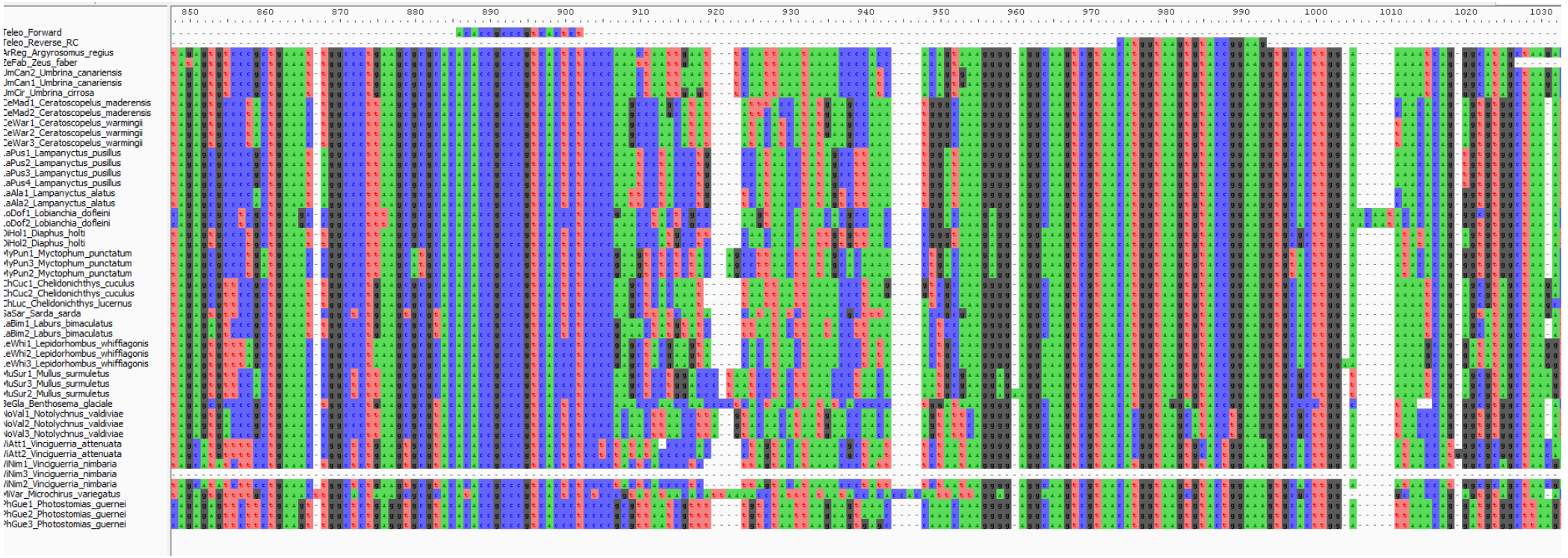

Figure 17. ClustalW multiple alignment of the obtained sequences and TELEO primer set.

Phylogenetic analysis

A total of 50 individuals were barcoded, belonging to 22 species and 19 genera. A genetic analysis of the specimens processed led to the following results.

Identity matrix between both specimens of *Ceratoscopelus maderiensis* was of 98%. Figure 18 shows the identity alignment for both individuals. The first row is the sequence of *Ceratoscopelus maderiensis*_1 whereas the second row is the sequence of *Ceratoscopelus maderiensis*_2. Dots represent that the base is the same. It can be seen a mismatch between C ant T bases at 874 ppb and 1050 ppb.

```
                ....|....| ....|....| ....|....| ....|....| ....|....|
                       860        870        880        890        900
ceratoscop1   CTCCCCAAGC CCAGCATATA TTTACCATAT GAAGCCAAAT GGGCAAAGGG
ceratoscop2   .......... .......... ..C....... .......... ..........

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       910        920        930        940        950
ceratoscop1   GAGGCAAGTC GTAACATGGT AAGTGTACCG GAAGGTGCAC TTGGACAACA
ceratoscop2   .......... .......... .......... .......... ..........

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       960        970        980        990       1000
ceratoscop1   CAGAGTGTGG CTAAATAGCA AAGCACCTCC CTTACACCGA GAAGATGTCC
ceratoscop2   .......... .......... .......... .......... ..........

                ....|....| ....|....| ....|....| ....|....| ....|....|
                      1010       1020       1030       1040       1050
ceratoscop1   GTGCAATTCG GACCACCCTG ATCCTAATAA GCTAGCCCGA CCCCTAAAAT
ceratoscop2   .......... .......... .......... .......... .........C
```

Figure 18. Plot identities for *Ceratoscopelus maderiensis* 1 and 2.

Identity matrix between specimens of *Ceratoscopelus warmingii* was of 97%. It can be seen a mismatch between C ant T bases at 311 ppb and 313 ppb *for Ceratoscopelus warmingii* 3 (figure 19). When calculating an identity matrix at genus level, it was scored a 94% of similarity between *Ceratoscopelus warmingii* and *Ceratoscopelus maderiensis.*

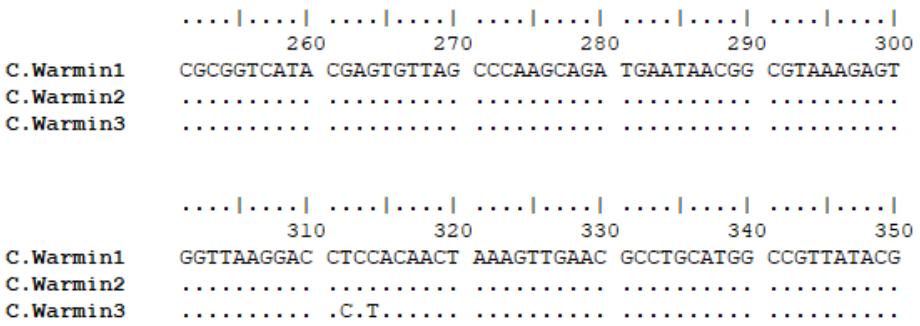

Figure 19. Plot identities for *Ceratoscopelus warmingii* 1, 2 and 3.

Identity matrix between both specimens of *Diaphus holti* was of 98%. It can be seen a mismatch between T and A bases at 209 ppb. Also, at 159 ppb there is a delected base for the second individual of *Diaphus holti* (Figure 20).

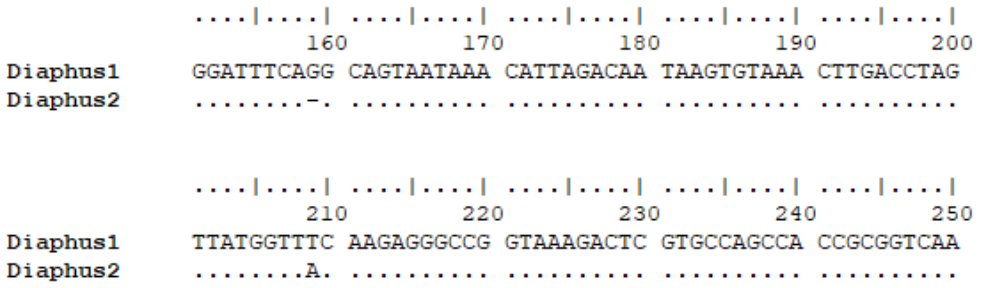

Figure 20. Plot identities for *Diaphus holti* 1 and 2.

Identity matrix between both specimens of *Lampanyctus alatus* was of 99%. It can be seen a mismatch between G and A bases at the 751 ppb. Also, a C has been changed for a T at 792 ppb (figure 21).

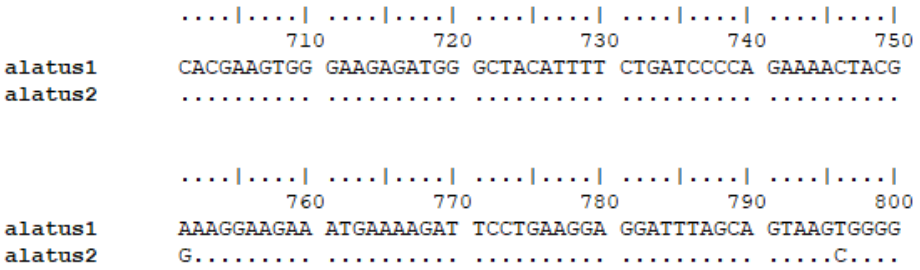

Figure 21. Plot identities for *Lampanyctus alatus* 1 and 2.

Identity matrix between the four specimens of *Lampanyctus pusillus* was of 98%. It is observed how a C base has been changed for a T base just for individuals 2 and 4, whereas individuals 1 and 3 are identical (figure 22). When calculating an identity matrix at genus level, it was scored a 94% of similarity between *Lampanyctus pusillus* and *Lampanyctus alatus.*

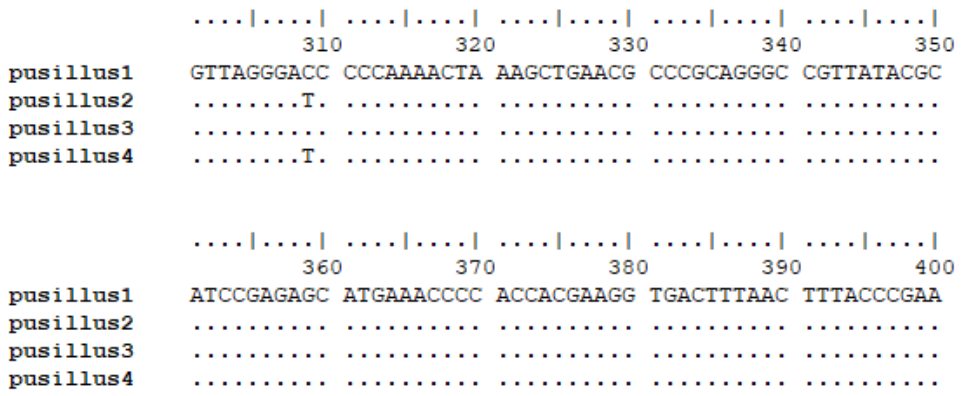

Figure 22. Plot identities for *Lampanyctus alatus* 1 and 2.

Identity matrix between the three specimens of *Mullus surmuletus* was of 98%. Two insertions of different bases are observed (figure 23) at the length of 903 ppb (A base) and at 1054 ppb (C base).

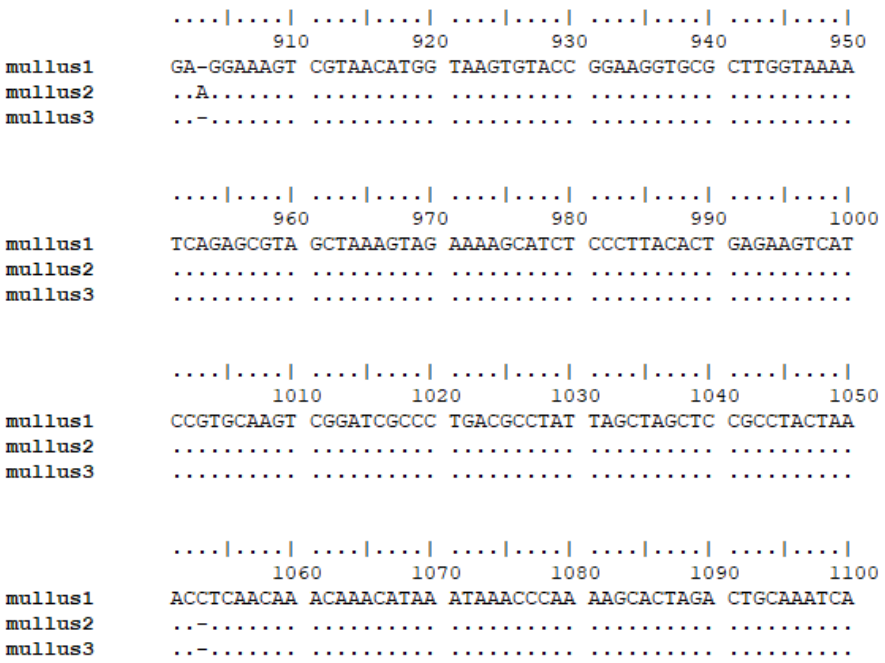

Figure 23. Plot identities for *Mullus surmuletus* 1, 2 and 3.

Identity matrix between the three specimens of *Notolychus valdiviae* was of 99%. A single change of a T base for a C is observed at 725 ppb (figure 24).

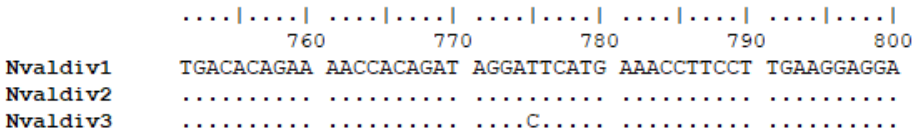

Figure 24. Plot identities for *Notolychus valdiviae* 1, 2 and 3.

Identity matrix between both specimens of *Umbrina canariensis* was of 99%, no significant changes are observed. When calculating an identity matrix at genus level, it was scored a 97% of similarity between *Umbrina cirrosa and Umbrina canariensis.* Moreover, similarity between *Vicinguerria attenuata* and *Vicinguerria nimbaria* is of 80% and no significant changes are observed among individuals of the same species.

The use of genetic distance as a reliable method of analysing barcode data was proposed by Herbert *et al*. (2003) and it is based on the belief that that interspecific divergence is greater than intraspecific divergence (Taylor and Harris, 2012). The results obtained agree with the latest statement; the similitude among same species ranks over 98% - 99% while, at genus level it drops down to 93%. Still, at genus level similarity is

greater than 90% except for *Vicinguerria* genus (80%). Interspecific divergence among *U. cirrosa* and *U. canariensis* is the lowest one (97%).

The Maximum likelihood tree (figure 25) also revealed closer phylogenetic relationship among same fish species. Individuals belonging to the same fish species were clustered under same nodes, also specimens of the same genus were nearer, confirming that they are closely related. Ideally, DNA barcodes should be specific enough to discriminate species (Pierce, 2019). As has been previously exposed, many studies remark the inability of COI gene to distinguish among very related species, advocating for the use of other genes (Cawthorn *et al.*, 2012; Deagle *et al.*, 2014; Pierce, 2019; Taylor and Harris, 2012), especially when applying it to metabarcoding studies (Pierce, 2019).

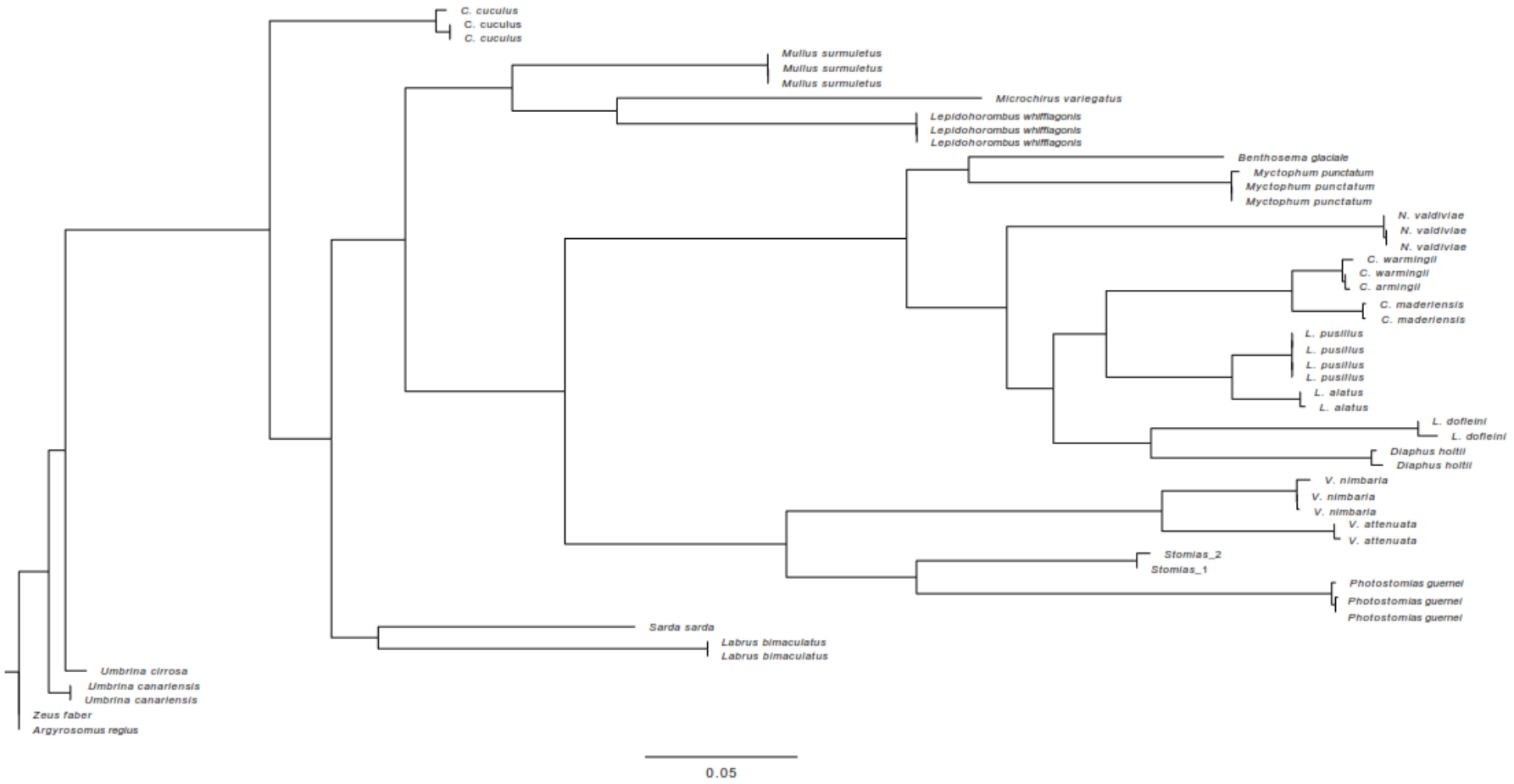

Figure 25. Maximum likelihood tree of 12s rRNA gene.

Concluding remarks

Our future depends on our ability to not taking from nature more than what it can offer us. A direct way to diagnosing environmental status is by documenting its biodiversity, but morphological based methods are outdated. Fast-evolving molecular techniques (DNA barcoding and metabarcoding) could enhance biodiversity research in the genomic era. In particular, it could be possible to extend spatial patterns of distribution across geographic regions or identify fragments of species. It also will allow a cost-effective means of discovering cryptic species and monitoring the endemic, endangered or invasive ones (Cristescu, 2014; Pawlowski *et al.,* 2018). Moreover, the capacity of detecting organisms present in the environment without causing any harm brings many ethical advantages, especially for the case of fish where classic samplings involve killing individuals (Pawlowski *et al*, 2020).

In contrast to traditional biomonitoring, where work of taxonomic expertise is required, DNA based approaches need molecular laboratories that can rapidly process a large number of samples (Figure 26). Data generated by next-generation biomonitoring differs from classic methods. For instance, metabarcoding cannot provide quantitative abundance information nor the age of individuals. This is not necessarily better or worse, but complementary. Analysis and interpretation of these data may require new statistical tools and advances in the field of bioinformatics (Figure 26) (Pawloski *et al*., 2020)

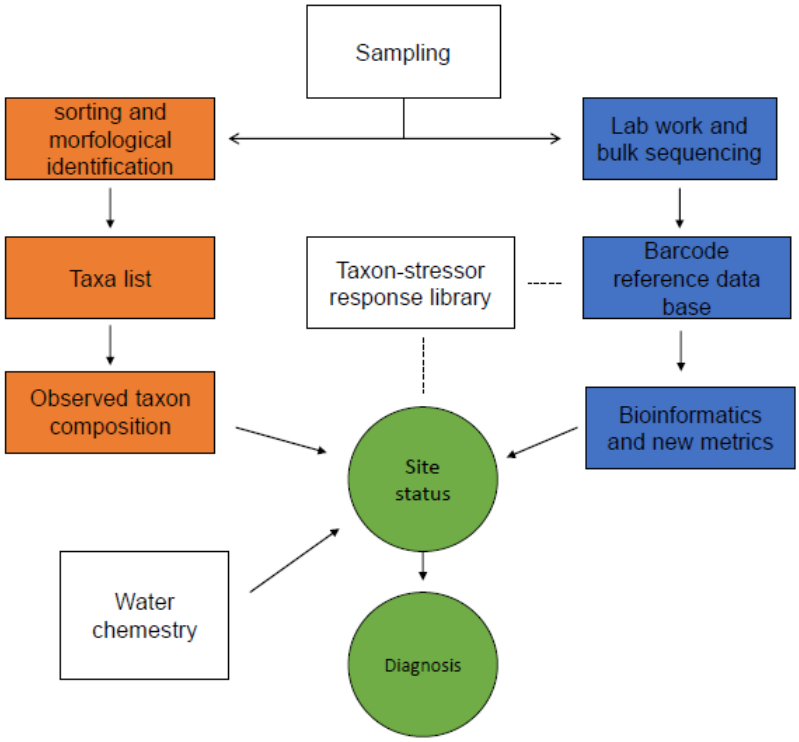

Figure 26. Traditional biomonitoring (*left;orange*) and DNA based biomonitoring (*right; blue*). An adaptation from the models proposed by Donald & Hajibabaei, (2012), and Lesse *et al*. (2018).

It has been stated that DNA barcoding is actually in the shadow of metabarcoding (Taylor and Harris, 2012). The latest is considered a promising technique as it has the potential to analyse a great number of individuals at a time, generating millions of sequences in one run, whereas DNA barcoding, generating just one sequence at a time, could be noticed as obsolete (Cristescu, 2014). However, through this work it is remarked the importance of combining both if reliable results want to be achieved. The lack of reference barcodes biases metabarcoding studies, preventing its implementation for biomonitoring. In a long-term framework, it is possible to imagine that an infrastructure for metabarcoding would be developed, as well as universal primers and markers. Robust catalogues of biodiversity would be provided thanks to the linking of reference libraries to morphological identified species (Cristescu, 2014).

CONCLUSIONS

Based on the objectives and from the results obtained it is concluded:

a) DNA was extracted from the tissue of marine fish samples. Some fish tissue samples are not suitable for DNA extraction possibly because of a non-adequate sample conservation. Overall, the DNA of samples was very degraded.

b) 12S PCR amplification completely failed for the following fish species: *Scorpaena scrofa, Valenciennellus tripunctulatus, Lepidophanes gaussi, Hygophum reinhardtii, Chauliodus danae, Chauliodus sloani* and *Molva macrophthalma*. It is not possible to determine the concrete cause of the PCR amplification failures, but thwo hypotheses had been proposed: I) the DNA is very degraded II) the primers elected are not universal. To accomplish the source of error, further trials need to be made.

c) Some sequences resulted in shorter fragments than others, probably due to a failure in the sequencing process due to low DNA concentration or low quality of the PCR products, which resulted in lack of Teleo region in some species.

d) A reference sequence of the 12S gene was obtained for the following fish species: *Argyrosomus regius, Benthosema glaciale, Ceratoscopelus maderiensis*, *Ceratoscopelus warmingii*, *Chelidonichthys cuculus*, *Chelidonichthys lucerns, Diaphus holti*, *Labrus bimaculatus*, *lampanyctus alatus*, *Lampanyctus pusillus*, *Lepidorombus whiffiagonis*, *Lobianchia dofleini*, *Microchirus variegatus*, *mullus surmuletus*, *Myctophum punctatum*, *Notolychnus valdiviae*, *Photostomias guernei*, *Sarda sarda , Stomias sp*, *Umbria canariensis*, *Umbria cirrosa, Viciguerria attenuate*, *Viciguerria nimbaria* and *Zeus faber*. Results are submitted to public access.

e) The Teleo region was identified in every sequence obtained except for one individual of *Vicinguerria nimbaria,* as has been exposed in conclusion c).

f) Interspecific divergence is greater than intraspecific divergence. the similitude among same species ranks over 98% - 99%. Single nucleotides changes have been found in: *Notolychus valdiviae, Mullus surmuletus, Lampanyctus alatus, Diaphus holti, Ceratoscopelus warmingii* and *Ceratoscopelus maderiensis,* The

greatest interspecific divergence was found in *Vicinguerria* genus (80%) and the lowes in *Umbrina* genus (97%).

Additional conclusions were delivered from the discussion exposed:

I. Genetic and molecular approaches hold a great promise for biomonitoring the marine ecosystem in the near future, but further developments need to be accomplished to confidently implement them.

II. Having a complete reference database is crucial to obtaining confident results through metabarcoding studies, as well as electing an appropriate marker.

III. The 12s rRNA gene seems to be more suitable for fish species discrimination than the COI gene.

IV. An integrative perspective between DNA barcoding, metabarcoding and taxonomic work is needed to confidently monitor marine areas.

FUTURE RESEARCH LINES

More exhaustive PCR trials need to be made to accomplishing the source of error. Marine Fish primers have been proven to amplify the target gene within a broad range of marine fish (Jin *et al*., 2013). However, for this project x fish species never amplified. It is still unclear whether primers operate for these specimens or not since this is the first attempt at sequencing them. Another approach could also be trying to amplify a different gene such as 16S or COI gene. It is feasible to imagine a future guideline that will eliminate the need of PCR steps. Indeed, (Zhou *et al.*, 2013) has demonstrated that this is possible.

This work aimed to increase the reference database, pretending therefore to fix the main challenge of metabarcoding studies. Although national and international barcoding campaigns had attempted to solve the lack of a reliable reference database, the coverage for marine species is still very low (Leese *et al.*, 2018), which hampers DNA based biomonitoring. The results obtained in this project are submitted to public access, which enables to anyone to use them as a reliable fish identification tool. DNA metabarcoding has shown to be a means by which marine resources could be surveyed without causing any negative impact. In this context, the present development will serve as a framework for future genetic studies, for fisheries management or for monitoring programmes.

Overall, I hope that this work will help to comprehend DNA based biomonitoring and identification methods and will contribute to their implementation.

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Copp, G. H., Geniez, P., Pont, D., Argillier, C., Baudoin, J. M., Peroux, T., Crivelli, A. J., Olivier, A., Acqueberge, M., Le Brun, M., Møller, P. R., Willerslev, E. and Dejean, T. (2016) Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding, *Molecular Ecology*. Blackwell Publishing Ltd, 25(4), pp. 929–942. doi: 10.1111/mec.13428.

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ANNEX I

>..\Sequences\FOR.ab1\.ab1\D10+70+For.ab1, ..\Sequences\REV.ab1\.ab1\D10+70+Rev.
GCCTGGGTCCTGACTTTATCATCCGCCCTAGCCAAATTTATACATGCAAGTCTCCGCACCCCTGTGAGAATGCCCTCAAA
CTCCTGCCCGGAAACGAGGAGCAGACATCAGGCACGCCAATCGCAGCCCAATACGTCTTGCTTAGCCACACCCCCAAGGG
AACTCAGCAGTAATAAACCTTAGGCAATAAGTGTAAACTCGACCTAGTTATGGCTACTAGAGGGCCGGTAAAACTCGTGC
CAGCCACCGCGGTCATACGAGTGTTAGCCCAAGCAGATGATAAACGGCGTAAAGAGTGGTTAGGGACCCCCAAAACTAAA
GTTGAACGCCTGCATGGCCGTTATACGCATCCGACGGCATGAAACCCCGCCACGAAAGTGACTTTAACCCAACCCGAACC
CACGACAGCTACGGCACAAACCGGGATTAGATACCCCGCTATGCTTAGTCGTAAACACCAATAGATAACTACAACTTCTA
TTCGCCTGGGGACTACTGGCACCAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTGTCCT
AGAACCGATATTCCCCGTTCGACCTCACCACTTCTAGCCCAACCCGCCTATATACCGCCGTCGTCAGCTTACCCCGTGAG
GGACAAGTAGTAAGCTAAACTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGCACGAAGTGTGCAAGAGATGGGCTA
CATTTTCTGACCCAGGAAACTACGAATGAAAGTATGAAAGTCTTTCTGAAGGCGGATTTAGCAGTAAGTAGGAAATAGAG
TGCCCTACTGAAACTGGCCCTTAAGCGCGCACACACCGCCCGTCACTCTCCCCAAGCCCAGCATATATTTACCATATGAA
GCCAAATGGGCAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGACAACACAGAGTGTGGCTA
AATAGCAAAGCACCTCCCTTACACCGAGAAGATGTCCGTGCAATTCGGACCACCCTGATCCTAATAAGCTAGCCCGACCC
CTAAAATGAACAACCCCCTTTAAAGTAGTTATGCAATAGAAAAATCAACAAACCATTTTTCCTCCCTAGTACCCGGGCGA
CAGAAAAGGA

Figure I. 1. 12S sequence of *Ceratoscopelus maderiensis*_1.

>..\Sequences\FOR.ab1\.ab1\D11+71+For.ab1, ..\Sequences\REV.ab1\.ab1\D11+71+Rev.
AAAGCCTGGGTCCTGACTTTATCATCCGCCCTAGCCAAATTTATACATGCAAGTCTCCGCACCCCTGTGAGAATGCCCTC
AAACTCCTGCCCGGAAACGAGGAGCAGACATCAGGCACGCCAATCGCAGCCCAATACGTCTTGCTTAGCCACACCCCCAA
GGGAACTCAGCAGTAATAAACCTTAGGCAATAAGTGTAAACTCGACCTAGTTATGGCTACTAGAGGGCCGGTAAAACTCG
TGCCAGCCACCGCGGTCATACGAGTGTTAGCCCAAGCAGATGATAAACGGCGTAAAGAGTGGTTAGGGACCCCCAAAACT
AAAGTTGAACGCCTGCATGGCCGTTATACGCATCCGACGGCATGAAACCCCGCCACGAAAGTGACTTTAACCCAACCCGA
ACCCACGACAGCTACGGCACAAACCGGGATTAGATACCCCGCTATGCTTAGTCGTAAACACCAATAGATAACTACAACTT
CTATTCGCCTGGGGACTACTGGCACCAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTGT
CCTAGAACCGATATTCCCCGTTCGACCTCACCACTTCTAGCCCAACCCGCCTATATACCGCCGTCGTCAGCTTACCCCGT
GAGGGACAAGTAGTAAGCTAAACTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGCACGAAGTGTGCAAGAGATGGG
CTACATTTTCTGACCCAGGAAACTACGAATGAAAGTATGAAAGTCTTTCTGAAGGCGGATTTAGCAGTAAGTAGGAAATA
GAGTGCCCTACTGAAACTGGCCCTTAAGCGCGCACACACCGCCCGTCACTCTCCCCAAGCCCAGCATATATTCACCATAT
GAAGCCAAATGGGCAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGACAACACAGAGTGTGG
CTAAATAGCAAAGCACCTCCCTTACACCGAGAAGATGTCCGTGCAATTCGGACCACCCTGATCCTAATAAGCTAGCCCGA
CCCCTAAAACGAACAACCCCCTTTAAAGTAGTTATGCAATAGAAAAATCAACAAACCATTTTTCCTCCCTAGTACCCGGG
CGAACAGAAAAGGAAATACC

Figure I. 2. 12S sequence of *Ceratoscopelus maderiensis*_2.

>..\Sequences\FOR.ab1\.ab1\D12+72+For.ab1, ..\Sequences\REV.ab1\.ab1\D12+72+Rev.
AAAGCCTGGGTCCTGACTTTATCATCCGCCCTAGCCAAATTTATACATGCAAGTCTCCGCACCCCCGTGAGAATGCCCTC
AAACTCCTGCCCGGAAACGAGGAGCAGACATCAGGCACGCCCGCCGCAGCCCAAGACGTCTTGCTTAGCCACACCCCCAA
GGGAACTCAGCAGTAATAAACCTTAGGCAATAAGTGTAAACTCGACCTAGTTATGGCTATTAGAGGGCCGGTAAAACTCG
TGCCAGCCACCGCGGTCATACGAGTGTTAGCCCAAGCAGATGAATAACGGCGTAAAGAGTGGTTAAGGACCTCCACAACT
AAAGTTGAACGCCTGCATGGCCGTTATACGCATCCGAAGGCATGAAACCCCACCACGAAAGTGACTTTAACCAGACCTGA
ACCCACGAAAGCTACGGCACAAACCGGGATTAGATACCCCGCTATGCTTAGTCGTAAACACCAATAGATAATTACAACTT
CTATTCGCCTGGGGACTACTGGCACCAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTGT
CCTAGAACCGATACTCCCCGTTCAACCTCACCACTTCTAGCCCAACCCGCCTATATACCGCCGTCGTCAGCTTACCCCGT
GAGGGACAAGTAGTAAGCTAAACTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGCACGAAGTGTGCAAGAGATGGG
CTACATTTTCTGACTCAGAAAACTACGGAAGAAAGTATGAAAGTCTTTCTGAAGGAGGATTTAGCAGTAAGTAGGTAATA
GAGTGCCCTACTGAAACTGGCCCTTAAGCGCGCACACACCGCCCGTCACTCTCCCCAAGCCCAACATATATACATCATAT
GAAGCCAAATGGGCAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGATAACACAGAGTGTGG
CTAAATAGTAAAGCACCTCCCTTACACCGAGAAGATGTCCGTGCAATTCGGACCACCCTGATCCTAATAAGCTAGCCCGA
CCGCTAAAATGAACAACCCCCTTTAAATTACTCATACTGTAAAAGAATCAACAAACCATTTTACCTCCCTAGTACACGGG
CGAACAGAAAAGGAAAACCGGAGCAA

Figure I. 3. 12S sequence of *Ceratoscopelus warmingii*_1.

>..\Sequences\FOR.ab1\.ab1\E1+73+For.ab1, ..\Sequences\REV.ab1\.ab1\E1+73+Rev.ab
TGGGTCCTGACTTTATCATCCGCCCTAGCCAAATTTATACATGCAAGTCTCCGCACCCCCGTGAGAATGCCCTCAAACTC
CTGCCCGGAAACGAGGAGCAGACATCAGGCACGCCCGCCGCAGCCCAAGACGTCTTGCTTAGCCACACCCCCAAGGGAAC
TCAGCAGTAATAAACCTTAGGCAATAAGTGTAAACTCGACCTAGTTATGGCTATTAGAGGGCCGGTAAAACTCGTGCCAG
CCACCGCGGTCATACGAGTGTTAGCCCAAGCAGATGAATAACGGCGTAAAGAGTGGTTAAGGACCTCCACAACTAAAGTT
GAACGCCTGCATGGCCGTTATACGCATCCGAAGGCATGAAACCCCACCACGAAAGTGACTTTAACCAGACCTGAACCCAC
GAAAGCTACGGCACAAACCGGGATTAGATACCCCGCTATGCTTAGTCGTAAACACCAATAGATAATTACAACTTCTATTC
GCCTGGGGACTACTGGCACCAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTGTCCTAGA
ACCGATACTCCCCGTTCAACCTCACCACTTCTAGCCCAACCCGCCTATATACCGCCGTCGTCAGCTTACCCCGTGAGGGA
CAAGTAGTAAGCTAAACTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGCACGAAGTGTGCAAGAGATGGGCTACAT
TTTCTGACTCAGAAAACTACGGAAGAAAGTATGAAAGTCTTTCTGAAGGAGGATTTAGCAGTAAGTAGGTAATAGAGTGC
CCTACTGAAACTGGCCCTTAAGCGCGCACACACCGCCCGTCACTCTCCCCAAGCCCAACATATATACATCATATGAAGCC
AAATGGGCAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGATAACACAGAGTGTGGCTAAAT
AGTAAAGCACCTCCCTTACACCGAGAAGATGTCCGTGCAATTCGGACCACCCTGATCCTAATAAGCTAGCCCGACCGCTA
AAATGAACAACCCCCTTTAAATTACTCATACTATAAAAGAATCAACAAACCATTTTACCTCCCTAGTACACGGGCGA

Figure I. 4. 12S sequence of *Ceratoscopelus warmingii*_2.

>..\Sequences\FOR.ab1\.ab1\E2+74+For.ab1, ..\Sequences\REV.ab1\.ab1\E2+74+Rev.ab
TCATCCGCCCTAGCCAAATTTATACATGCAAGTCTCCGCACCCCCGTGAGAATGCCCTCAAACTCCTGCCCGGAAACGAG
GGGCAGACATCAGGCACGCCCGCCGCAGCCCAAGACGTCTTGCTTAGCCACACCCCCAAGGGAACTCAGCAGTAATAAAC
CTTAGGCAATAAGTGTAAACTCGACCTAGTTATGGCTATTAGAGGGCCGGTAAAACTCGTGCCAGCCACCGCGGTCATAC
GAGTGTTAGCCCAAGCAGATGAATAACGGCGTAAAGAGTGGTTAAGGACCCCTACAACTAAAGTTGAACGCCTGCATGGC
CGTTATACGCATCCGAAGGCATGAAACCCCACCACGAAAGTGACTTTAACCAGACCTGAACCCACGAAAGCTACGGCACA
AACCGGGATTAGATACCCCGCTATGCTTAGTCGTAAACACCAATAGATAATTACAACTTCTATTCGCCTGGGGACTACTG
GCACCAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTGTCCTAGAACCGATACTCCCCGT
TCAACCTCACCACTTCTAGCCCAACCCGCCTATATACCGCCGTCGTCAGCTTACCCCGTGAGGGACAAGTAGTAAGCTAA
ACTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGCACGAAGTGTGCAAGAGATGGGCTACATTTTCTGACTCAGAAA
ACTACGGAAGAAAGTATGAAAGTCTTTCTGAAGGAGGATTTAGCAGTAAGTAGGTAATAGAGTGCCCTACTGAAACTGGC
CCTTAAGCGCGCACACACCGCCCGTCACTCTCCCCAAGCCCAACATATATACATCATATAAAGCCAAATGGGCAAAGGGG
AGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGATAACACAGAGTGTGGCTAAATAGTAAAGCACCTCCC
TTACACCGAGAAGATGTCCGTGCAATTCGGACCACCCTGATCCTAATAAGCTAGCCCGACCGCTAAAATGAACAACCCCC
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Figure I. 5. 12S sequence of *Ceratoscopelus warmingii*_3.

>..\Sequences\FOR.ab1\.ab1\B11+24+For.ab1, ..\Sequences\SEQUENCING_2\F8+24r+Reve
AGGCTTGGGTCCTGACTTTACTATCAACTTTAGCTAAACTTACACATGCAAGTATCCGCACCCCTGTGAGAATGCCCTAC
AGTTCCCCGCCCGGGAACGAGGAGCCGGTATCAGGCACACACAACCAGCCCATGACACCTTGCTTAGCCACACCCTCAAG
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CAGCCACCGCGGTTATACGAGAGGCCCAAGTTGACAGTCACCGGCGTAAAGAGTGGTTAAAGAATGATTAAAACTAAAGC
CGAACACCTTCAAGGCAGTTATACGCACCCGAAGGTTAGAAGCCCAACTACGAAAGTGGCTTTATCTTTCCTGAACCCAC
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GCCCGGGAACTACGAGCATCAGCTTGAAACCCAAAGGACTTGGCGGTGCTTTAGATCCACCTAGAGGAGCCTGTTCTAGA
ACCGATAACCCCCGTTCAACCTCACCTTTTCTTGTTTTCCCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGA
CTCATAGTAAGCAAAATTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGTATGGAAAGGGAAGAAATGGGCTACATT
CCCTATAATTAGTGAATACGGACGATGTCCTGAAAGAGACATCTGAAGGAGGATTTAGCAGTAAGCAGGAAATAGAGCGT
TCCGCTGAAATTGGCCCTGAAGCGCGCACACACCGCCCGTCACTCTCCCCAAGCTCACAAATTAATTAATTAAAACCCTA
AGGTCGCAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAAAAATCAGAGCGTAGCTAAGAC
AGAAAAGCATCTCCCTTACACTGAGAAGTCGCCCGTGCAAATCGGACCGCCCTGATGCTTAACAGCTAGCCCACCCAACC
AACAACAACAACCCCCTATAAATACCCCCAAATAAACAAGTATTAACCAAACAAACCATTTTCCCCCTAAGTATGGGCGA
CAGAAAAGGACCT

Figure I. 6. 12S sequence of *Chelidonichthys cuculus_1.*

>..\Sequences\FOR.ab1\.ab1\B10+23+For.ab1, ..\Sequences\SEQUENCING_2\F8+24r+Reve
AAGGCTTGGGTCCTGACTTTACTATCAACTTTAGCTAAACTTACACATGCAAGTATCCGCACCCCTGTGAGAATGCCCTA
CAGTTCCCCGCCCGGGAACGAGGAGCCGGTATCAGGCACACACAACCAGCCCATGACACCTTGCTTAGCCACACCCTCAA
GGGAACTCAGCAGTGATAGACATTAAGCCATAAGTGAAAACTTGACTTAGTCAAAGCTAACAGGGCCGGTAAAACTCGTG
CCAGCCACCGCGGTTATACGAGAGGCCCAAGTTGACAGTCACCGGCGTAAAGAGTGGTTAAAGAATGATTAAAACTAAAG
CCGAACACCTTCAAGGCAGTTATACGCACCCGAAGGTTAGAAGCCCAACTACGAAAGTGGCTTTATCTTTCCTGAACCCA
CGAGAGCTACGGCACAAACTGGGATTAGATACCCCACTATGCCTAGCCCTAAACATTGATAGTACTCTACACCCACTATC
CGCCCGGGAACTACGAGCATCAGCTTGAAACCCAAAGGACTTGGCGGTGCTTTAGATCCACCTAGAGGAGCCTGTTCTAG
AACCGATAACCCCCGTTCAACCTCACCTTTTCTTGTTTTCCCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGG
ACTCATAGTAAGCAAAATTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGTATGGAAAGGGAAGAAATGGGCTACAT
TCCCTATAATTAGTGAATACGGACGATGTCCTGAAAGAGACATCTGAAGGAGGATTTAGCAGTAAGCAGGAAATAGAGCG
TTCCGCTGAAATTGGCCCTGAAGCGCGCACACACCGCCCGTCACTCTCCCCAAGCTCACAAATTAATTAATTAAAACCCT
AAGGTCGCAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAAAAATCAGAGCGTAGCTAAGA
CAGAAAAGCATCTCCCTTACACTGAGAAGTCGCCCGTGCAAATCGGACCGCCCTGATGCTTAACAGCTAGCCCACCCAAC
CAACAACAACAACCCCCTATAAATACCCCCAAATAAACAAGTATTAACCAAACAAACCATTTTCCCCCTAAGTATGGGCG
ACAGAAAAGGACC

Figure I. 7. 12S sequence of *Chelidonichthys cuculus_2.*

>..\Sequences\FOR.ab1\.ab1\A10+11+For.ab1, ..\Sequences\REV.ab1\.ab1\A10+11+Rev.
AAAGGCTTGGGTCCTGACTTTACTATCAACTTTAGCTAAACTTACACATGCAAGTATCCGCACCCCTGTGAGAATGCCCT
ACAGTTCCCCGCCCGGGAACAAGGAGCCGGTATCAGGCACACATAACCCAGCCCATGACACCTTGCTTAGCCACACCCTC
AAGGGAACTCAGCAGTGATAGACATTAAGCCATAAGTGAAAACTTGACTTAGTCAAAGCTAACAGGGCCGGTAAAACTCG
TGCCAGCCACCGCGGTTATACGAGAGGCCCAAGTTGACAGTCACCGGCGTAAAGAGTGGTTAAAGAATGATTAAAACTAA
AGCCGAACACCTTCAAGGCAGTTATACGCACCCGAAGGTTAGAAGCCCAACTACGAAAGTGGCTTTATCTTTCCTGAACC
CACGAGAGCTACGGCACAAACTGGGATTAGATACCCCACTATGCCTAGCCCTAAACATTGATAGTACTCTACACCCACTA
TCCGCCCGGGAACTACGAGCATCAGCTTGAAACCCAAAGGACTTGGCGGTGCTTTAGATCCACCTAGAGGAGCCTGTTCT
AGAACCGATAACCCCCGTTCAACCTCACCTTTTCTTGTTTTCCCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAA
GGACTCATAGTAAGCAAAATTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGTATGGAAAGGGAAGAAATGGGCTAC
ATTCCCTATAATTAGTGAATACGGACGATGTCCTGAAAGAGACATCTGAAGGAGGATTTAGCAGTAAGCGGGAAATAGAG
CGTTCCGCTGAAATTGGCCCTGAAGCGCGCACACACCGCCCGTCACTCTCCCCAAGCTCACAAATTAATTAATTAAAACC
CTAAAATCGCAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAAAAATCAGAGCGTAGCTAA
GCCAGAAAAGCATCTCCCTTACACTGAGAAGTCGCCCGTGCAAATCGGACCGCCCTGATGCTTAACAGCTAGCCCACCCA
ACCAACAACAACAACCCCCTATTAATACCCCCAAATAAACAAGTATCAACCAAACAAACCATTTTCCCCCTTAGTATGGC
GACAGAAAAGGA

Figure I. 8. 12S sequence of *Chelidonichthys lucernus.*

>..\Sequences\SEQUENCING_2\F12+77r+Reverse.ab1, ..\Sequences\SEQUENCING_2\H8+77f
TCCTGACTTTGTCATCCGCCTTAACCAAATTTATACATGCAAGTATCCGCATCCCCGTGAAAATGCCCCTAAAATCCTGC
CCGGAAACGAGGAGCAGACATCAGGCACACAAGTGTAGCCCACGACGTCTTGCACAGCCACACCCCCAAGGGATTTCAGG
CAGTAATAAACATTAGACAATAAGTGTAAACTTGACCTAGTTATGGTTTCAAGAGGGCCGGTAAAGACTCGTGCCAGCCA
CCGCGGTCAAACGAGGTAGTGCCCGAGCAGATGACCAACGGCGTAAAGAGTGGTTAGGGAACCCCAAAACTAAAGCTAAA
CGCCCACAGGGCTGTGATACGCATCCGATGGTATGAGACCCCGCCACGAAAGTGACTTTAACCAACCCGAACCCACGACA
GCCAGGCCACAAACTGGGATTAGATACCCCACTATGCCTAGCCTTAAACATTGATAGAAACGCCACAAATTCTATCCGCC
CGGGTACTACAAGCATTAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTGTCCTAGAACC
GATACCCCCCGTTCAACCTCACCACTTCTGGCTCAATACTCCGCCTGTATACCTCCGTCGTCAGCTTACCCCGTGAGGGA
ATCATAGTTAGCTAAATTGGCACACCCCCAAAACGTCAGGTCAAGGTGCAGCGCACGAAGTGTGGAAGAGATGGGCTACA
TTTTCTGGACCAGAAAATTACGTAAGGGGCAAATGAAAAGACCCCTAAAGGAGGATTTAGCAGTAAGCAAGGAATAGAGT
GCCCTGCTGAAATTGGCCCTTAAGCGCGCACACACCGCCCGTCACTCTCCCCAAACCCATGCCTTCAACAACATATTGTG
TTAACCGGGTAAAGAGGAGGAAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCGCTTGGAATATACAGAGTGTGGCTAA
ATAGCAAAGCACCTCCCTTACACTGAGAAGATGTCCGTGCAATTCGGACCACCCTGATCCCAAAAAGCTAGCCCAACCCC
CCCCGATAAAGCACAACCTTTAAAACTAATCCCTGTAAAACACCCAACAAACCATTTTACCTCCCCAGTACCCGGGCGAC
AGAAAAGGAG

Figure I. 9. 12S sequence of *Diaphus holti_1.*

>..\Sequences\SEQUENCING_2\D1+76f+Forward.ab1, ..\Sequences\SEQUENCING_2\H1+76r+
TCCTGACTTTGTCATCCGCCTTAACCAAATTTATACATGCAAGTATCCGCATCCCCGTGAAAATGCCCCTAAAATCCTGC
CCGGAAACGAGGAGCAGACATCAGGCACACAAGTGTAGCCCACGACGTCTTGCACAGCCACACCCCCAAGGGATTTCAGC
AGTAATAAACATTAGACAATAAGTGTAAACTTGACCTAGTTATGGTTACAAGAGGGCCGGTAAAGACTCGTGCCAGCCAC
CGCGGTCAAACGAGGTAGTGCCCGAGCAGATGACCAACGGCGTAAAGAGTGGTTAGGGAACCCCAAAACTAAAGCTAAAC
GCCCACAGGGCTGTGATACGCATCCGATGGTATGAGACCCCGCCACGAAAGTGACTTTAACCAACCCGAACCCACGACAG
CCAGGCCACAAACTGGGATTAGATACCCCACTATGCCTAGCCTTAAACATTGATAGAAACGCCACAAATTCTATCCGCCC
GGGTACTACAAGCATTAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTGTCCTAGAACCG
ATACCCCCCGTTCAACCTCACCACTTCTGGCCCAATACTCCGCCTGTATACCTCCGTCGTCAGCTTACCCCGTGAGGGAA
TCATAGTTAGCTAAATTGGCACACCCCCAAAACGTCAGGTCAAGGTGCAGCGCACGAAGTGTGGAAGAGATGGGCTACAT
TTTCTGGACCAGAAAATTACGTAAGGGGCAAATGAAAAGACCCCTAAAGGAGGATTTAGCAGTAAGCAAGGAATAGAGTG
CCCTGCTGAAATTGGCCCTTAAGCGCGCACACACCGCCCGTCACTCTCCCCAAACCCATGCCTCCAACAACATATTGTGT
TAACCGGGTAAAGAGGAGGAAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCGCTTGGAATATACAGAGTGTGGCTAAA
TAGCAAAGCACCTCCCTTACACTGAGAAGATGTCCGTGCAATTCGGACCACCCTGATCCCAAAAAGCTAGCCCAACCCCC
CGATAAAGCACACCTTTAAAACTAATCCCTGTAAAACACCCAACAAACCATTTTACCTCCCCAGTACCCGGGCGACAGAA
AAAGGAAACC

Figure I. 10. 12S sequence of *Diaphus holti_2.*

>..\Sequences\FOR.ab1\.ab1\C7+36+For.ab1, ..\Sequences\REV.ab1\.ab1\C7+36+Rev.ab
AAAGGCTTGGGTCCTGACTTTGTTATCAACTTTAGCTAGACTTACACATGCAAGCATCCGCACTCCAGTGAGAATGCCCT
ATGGTCCTCTGTTTGAGGGCTGGGAGCTGGTATCAGGCACAAGAACTTAGCCCACGACGCCTTGTTTAGCCACACCCTCA
AGGGAATTCAGCAGTGATAAACATTAAGCCATAAGTGAAAACTTGACTTAGTTAAAGCTAAGAGGGCCGGTAAATCTCGT
GCCAGCCACCGCGGTTATACGAAAGACCCAAGTTGATAGTCACCGGCGTAAAGAGTGGTTAAGATAAATCCACAAACTAA
AGCCGAACATCTTCAAAGCTGTTATACGCACACGAAGAAAGGAAATCCAACCACGAAAGTGGCTTTATATCATCTGACCC
CACGAAAGCTATGATACAAACTGGGGATTAGATACCCCACTATGCTTAGCCGTAAACATTGATAGTACAGTACATCTACT
ATCCGCCCGGGTACTACGAGCATAAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGATCCACCTAGAGGAGCCTGTTC
TAGAACCGATAATCCCCGTTAAACCTCACCTTTTCTTGTTTTCCCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGA
AGGGTCAATAGTAAGCAAAATTGGCATAGCCCAAAACGCCAGGTCGAGGTGTAGCGTATGAAAAGGGAAGAAATGGGCTA
CATTCAATATAACAATGCATACGGATGGTGGCCTGAAATACCCACCTGAAGGAGGATTTAGCAGTAAGCAGGAAATAGAG
AGTCCCGCTGAAATTGGCCCTGAAGCGCGTACACACCGCCCGTCACTCTCCCCGAAACTATGTATCTTAATACTTAATAC
CTTAAAACTCCAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAAAAATCAGAGCATAGCTA
AATAACGAATAGCACCTCCCTTACACCGAGAAGATACCCGTGCAACCCGGGTTGCCCTGACACCCAATAGCTAGCCCACC
CCCATAAAATCAACAAATCAACATTTATAACCCCTCATCCCCGAAACCTAAAATTAACAAACCATTTTCCACCTCAGTAT
AGGCGATAGAAAAGG

Figure I. 11. 12S sequence of *Labrus bimaculatus_1.*

>..\Sequences\REV.ab1\.ab1\C8+37+Rev.ab1, ..\Sequences\SEQUENCING_2\D3+37f+Forwa
GCACAAAGGCTTGGGTCCTGACTTTGTTATCAACTTTAGCTAGACTTACACATGCAAGCATCCGCACTCCAGTGAGAATG
CCCTATGGTCCTCTGTTTGAGGGCTGGGAGCTGGTATCAGGCACAAGAACTTAGCCCACGACGCCTTGTTTAGCCACACC
CTCAAGGGAATTCAGCAGTGATAAACATTAAGCCATAAGTGAAAACTTGACTTAGTTAAAGCTAAGAGGGCCGGTAAATC
TCGTGCCAGCCACCGCGGTTATACGAAAGACCCAAGTTGATAGTCACCGGCGTAAAGAGTGGTTAAGATAAATCCACAAA
CTAAAGCCGAACATCTTCAAAGCTGTTATACGCACACGAAGAAAGGAAATCCAACCACGAAAGTGGCTTTATATCATCTG
ACCCCACGAAAGCTATGATACAAACTGGGATTAGATACCCCACTATGCTTAGCCGTAAACATTGATAGTACAGTACATCT
ACTATCCGCCCGGGTACTACGAGCATAAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGATCCACCTAGAGGAGCCTG
TTCTAGAACCGATAATCCCCGTTAAACCTCACCTTTTCTTGTTTTCCCCGCCTATATACCGCCGTCGTCAGCTTACCCTG
TGAAGGGTCAATAGTAAGCAAAATTGGCATAGCCCAAAACGCCAGGTCGAGGTGTAGCGTATGAAAAGGGAAGAAATGGG
CTACATTCAATATAACAATGCATACGGATGGTGGCCTGAAATACCCACCTGAAGGAGGATTTAGCAGTAAGCAGGAAATA
GAGAGTCCCGCTGAAATTGGCCCTGAAGCGCGTACACACCGCCCGTCACTCTCCCCGAAACTATGTATCTTAATACTTAA
TACCTTAAAACTCCAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAAAAATCAGAGCATAG
CTAAATAACGAATAGCACCTCCCTTACACCGAGAAGATACCCGTGCAACCCGGGTTGCCCTGACACCCAATAGCTAGCCC
ACCCCCATAAAATCAACAAATCAACATTTATAACCCCTCATCCCCGAAACCTAAAATTAACAAACCATTTTTCCACCTCA
GTATAGGCGATAGAAAAGGAG

Figure I. 12. 12S sequence of *Labrus bimaculatus_2.*

>..\Sequences\SEQUENCING_2\C5+98f+Forward.ab1, ..\Sequences\SEQUENCING_2\G5+98r+
AAAGCCTGGGTCCTGACTTTACCATCCGCCCCAACCAGATTTATACATGCAAGTATCCGCATCCCCGTGAGAATGCCCCT
AAAATCCTGCCCGGAAACGAGGAGCAGACATCAGGCACGCCCGCGCAGCCCAAGACGTCTTGCTTAGCCACACCCCCAAG
GGAACTCAGCAGTAATAAACTTTAGACCATAAGTGAAAACTTGATCTAGTTATGGTTGTCAGAGGGCCGGTAAAACTCGT
GCCAGCCACCGCGGTCATACGAGTGTTAGCCCAAGTGGATGGTCAACGGCGTAAAGAGTGGTTAGGGGCCTCCAAAACTA
AAGCTGAACGCCCTTCCGGGCTGTTATACGCATCCGAGAGCATGAAACCCTGCCACGAAGGTGGCTTTAACCTTACCCGA
ACCCACGACAGCTAGGGCACAAACTGGGATTAGATACCCCACTATGCCTAGTCGTAAACCTTAATAGAAACTGCACCCAT
TCTATTCGCCAGGGTACTACAAGCACTAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTG
TCCTAGAACCGATACCCCCCGTTCAACCTCACCACTTCTAGCCCAAACCGCCTATATACCACCGTCGTCAGCTTACCCCG
TGAGGGACTAATAGTAAGCTAAACTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGCACGAAGTGGGAAGAGATGGG
CTACATTTTCTGATCCCCAGAAAACTACGAAAGGAAGAAATGAAAAGATTCCTGAAGGAGGATTTAGCAGTAAGTGGGGA
ATAGAGCGCCCCACTGAAATAGGCCCTTAAGCGCGCACACACCGCCCGTCACTCTCCCCAATTCCTACCCTGTCATAACC
TATAGTCTTAAATGGATAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGACAACACAGGGTG
TGGCTAAATAGCAAAAGCACCTCCCTTACACCGAGAAGATGTCCGTGCAATTCGGACCACCCTGATCCTAAAAAGCTAGC
CCGCCCCTTAGAGAAACAACCCCCTCTAAATTAAATTACCCATGAAACAAGCAACAAACCATTTTACTTCCCCAGTACCC
GGGCGACAGAAAAGGAAAACC

Figure I. 13. 12S sequence of *Lampanyctus alatus_1.*

>..\Sequences\SEQUENCING_2\C11+99f+Forward.ab1, ..\Sequences\SEQUENCING_2\F11+99
AAAAGCCTGGGTCCTGACTTTACCATCCGCCCCAACCAGATTTATACATGCAAGTATCCGCATCCCCGTGAGAATGCCCC
TAAAATCCTGCCCGGAAACGAGGAGCAGACATCAGGCACGCCCGCGCAGCCCAAGACGTCTTGCTTAGCCACACCCCCAA
GGGAACTCAGCAGTAATAAACTTTAGACCATAAGTGAAAACTTGATCTAGTTATGGTTGTCAGAGGGCCGGTAAAACTCG
TGCCAGCCACCGCGGTCATACGAGTGTTAGCCCAAGTGGATGGTCAACGGCGTAAAGAGTGGTTAGGGGCCTCCAAAACT
AAAGCTGAACGCCCTCCGGGCTGTTATACGCATCCGAGAGCATGAAACCCTGCCACGAAGGTGGCTTTAACCTTACCCGA
ACCCACGACAGCTAGGGCACAAACTGGGATTAGATACCCCACTATGCCTAGTCGTAAACCTTAATAGAAACTGCACCCAT
TCTATTCGCCAGGGTACTACAAGCACTAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTG
TCCTAGAACCGATACCCCCCGTTCAACCTCACCACTTCTAGCCCAAACCGCCTATATACCACCGTCGTCAGCTTACCCCG
TGAGGGACTAATAGTAAGCTAAACTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGCACGAAGTGGGAAGAGATGGG
CTACATTTTCTGATCCCCAGAAAACTACGGAAGGAAGAAATGAAAAGATTCCTGAAGGAGGATTTAGCAGTAAGCGGGGA
ATAGAGCGCCCCACTGAAATAGGCCCTTAAGCGCGCACACACCGCCCGTCACTCTCCCCAATTCCTACCCTGTCATAACC
TATAGTCTTAAATGGATAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGACAACACAGGGTG
TGGCTAAATAGCAAAGCACCTCCCTTACACCGAGAAGATGTCCGTGCAATTCGGACCACCCTGATCCTAAAAAGCTAGCC
CGCCCCTTAGAGAAACAACCCCCTCTAAATTAAATTACCCATGAAACAAGCAACAAACCCATTTTACTTCCCCAGTACCC
GGGCGACAGAAAAGGAA

Figure I. 14. 12S sequence of *Lampanyctus alatus_2.*

>..\Sequences\SEQUENCING_2\C3+106f+Forward.ab1, ..\Sequences\SEQUENCING_2\G3+106
AAAGCCTGGGTCCTGACTTTACCATCCGCCCCAGCCAGATTTATACATGCAAGTATCCGCACCCCCGTGAGAATGCCCCT
AAGATCCTGCCCGGAAATGAGGAGCAGACATCAGGCACGCCCACGCAGCCCAAGACGTCTTGCTTAGCCACACCCCCAAG
GGAACTCAGCAGTAATAAACTTTAGACCATGAGTGTAAACTTGATCTAGTTATGGTTATAAGAGGGCCGGTAAAACTCGT
GCCAGCCACCGCGGTCATACGAGTGTTAGCCCAAGTGGATGGTTAACGGCGTAAAGAGTGGTTAGGGACCCCCAAAACTA
AAGCTGAACGCCCGCAGGGCCGTTATACGCATCCGAGAGCATGAAACCCCACCACGAAGGTGACTTTAACTTTACCCGAA
CCCACGACAGCTAGGGTACAAACTGGGATTAGATACCCCACTATGCCTAGTCGTAAACTTTAATAGAAGCCGCACCCCCT
CTATTCGCCAGGGCACTACGAGCACCAGCTTGAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTGT
CCTAGAACCGATACCCCCCGTTCAACCTCACCACTTCTAGCCCAAACCGCCTATATACCACCGTCGTCAGCTTACCCCGT
GAGGGACTAATAGTAAGCTAAACTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGCACGAAGTGGGAAGAGATGGGC
TACATTTTCTGACCCAGAAAACTACGGAAGGAAAAATGAAAAGATTCCTGAAGGAGGATTTAGCAGTAAGCAGGGAGTAG
AGCGCCCCGCTGAAATAGGCCCTTAAGCGCGCACACACCGCCCGTCACTCTCCCCAAATCCTACCCTGCCATAACCTATA
GCCTTAAATGGATAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAAAACACAGTGTGTGGC
TAAATAGTAAAGCACCTCCCTTACACCGAGAAGATGTCCGTGCAATTCGGACCACCCTGATTCTAAAAAACTAGCCCGCC
CCTTAGAGAAACAACCCCCTCTAAATTATATTACCCGCACTACAAGCAACAAACCATTTTACTTCCCCAGTACCCGGGCG
ACAGAAAAGGAA

Figure I. 15. 12S sequence of *Lampanyctus pusillus_1.*

>..\Sequences\FOR.ab1\.ab1\F6+104+For.ab1, ..\Sequences\REV.ab1\.ab1\F6+104+Rev.
AAGCCTGGGTCCTGACTTTACCATCCGCCCCAGCCAGATTTATACATGCAAGTATCCGCACCCCCGTGAGAATGCCCCTA
AGATCCTGCCCGGAAATGAGGAGCAGACATCAGGCACGCCCACGCAGCCCAAGACGTCTTGCTTAGCCACACCCCCAAGG
GAACTCAGCAGTAATAAACTTTAGACCATGAGTGTAAACTTGATCTAGTTATGGTTATAAGAGGGCCGGTAAAACTCGTG
CCAGCCACCGCGGTCATACGAGTGTTAGCCCAAGTGGATGGTTAACGGCGTAAAGAGTGGTTAGGGATCCCCAAAACTAA
AGCTGAACGCCCGCAGGGCCGTTATACGCATCCGAGAGCATGAAACCCCACCACGAAGGTGACTTTAACTTTACCCGAAC
CCACGACAGCTAGGGTACAAACTGGGATTAGATACCCCACTATGCCTAGTCGTAAACTTTAATAGAAGCCGCACCCCCTC
TATTCGCCAGGGCACTACGAGCACCAGCTTGAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTGTC
CTAGAACCGATACCCCCCGTTCAACCTCACCACTTCTAGCCCAAACCGCCTATATACCACCGTCGTCAGCTTACCCCGTG
AGGGACTAATAGTAAGCTAAACTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGCACGAAGTGGGAAGAGATGGGCT
ACATTTTCTGACCCAGAAAACTACGGAAGGAAAAATGAAAAGATTCCTGAAGGAGGATTTAGCAGTAAGCAGGGAGTAGA
GCGCCCCGCTGAAATAGGCCCTTAAGCGCGCACACACCGCCCGTCACTCTCCCCAAATCCTACCCTGCCATAACCTATAG
CCTTAAATGGATAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAAAACACAGTGTGTGGCT
AAATAGTAAAGCACCTCCCTTACACCGAGAAGATGTCCGTGCAATTCGGACCACCCTGATTCTAAAAAACTAGCCCGCCC
CTTAGAGAAACAACCCCCTCTAAATTATATTACCCGCACTACAAGCAACAAACCATTTTACTTCCCCAGTACCCGGGCGA
CAGAAAAGGA

Figure I. 16. 12S sequence of *Lampanyctus pusillus_2.*

>..\Sequences\FOR.ab1\.ab1\F8+106+For.ab1, ..\Sequences\REV.ab1\.ab1\F8+106+Rev.
GGTCCTGACTTTACCATCCGCCCCAGCCAGATTTATACATGCAAGTATCCGCACCCCCGTGAGAATGCCCCTAAGATCCT
GCCCGGAAATGAGGAGCAGACATCAGGCACGCCCACGCAGCCCAAGACGTCTTGCTTAGCCACACCCCCAAGGGAACTCA
GCAGTAATAAACTTTAGACCATGAGTGTAAACTTGATCTAGTTATGGTTATAAGAGGGCCGGTAAAACTCGTGCCAGCCA
CCGCGGTCATACGAGTGTTAGCCCAAGTGGATGGTTAACGGCGTAAAGAGTGGTTAGGGACCCCCAAAACTAAAGCTGAA
CGCCCGCAGGGCCGTTATACGCATCCGAGAGCATGAAACCCCACCACGAAGGTGACTTTAACTTTACCCGAACCCACGAC
AGCTAGGGTACAAACTGGGATTAGATACCCCACTATGCCTAGTCGTAAACTTTAATAGAAGCCGCACCCCCTCTATTCGC
CAGGGCACTACGAGCACCAGCTTGAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTGTCCTAGAAC
CGATACCCCCCGTTCAACCTCACCACTTCTAGCCCAAACCGCCTATATACCACCGTCGTCAGCTTACCCCGTGAGGGACT
AATAGTAAGCTAAACTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGCACGAAGTGGGAAGAGATGGGCTACATTTT
CTGACCCAGAAAACTACGGAAGGAAAAATGAAAAGATTCCTGAAGGAGGATTTAGCAGTAAGCAGGGAGTAGAGCGCCCC
GCTGAAATAGGCCCTTAAGCGCGCACACACCGCCCGTCACTCTCCCCAAATCCTACCCTGCCATAACCTATAGCCTTAAA
TGGATAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAAAACACAGTGTGTGGCTAAATAGT
AAAGCACCTCCCTTACACCGAGAAGATGTCCGTGCAATTCGGACCACCCTGATTCTAAAAAACTAGCCCGCCCCTTAGAG
AAACAACCCCCTCTAAATTATATTACCCGCACTACAAGCAACAAACCATTTACTTCCCCAGTACCCGGGCGACAGAAAA

Figure I. 17. 12S sequence of *Lampanyctus pusillus_3.*

>..\Sequences\FOR.ab1\.ab1\F7+105+For.ab1, ..\Sequences\REV.ab1\.ab1\F7+105+Rev.
AGCCTGGGTCCTGACTTTACCATCCGCCCCAGCCAGATTTATACATGCAAGTATCCGCACCCCCGTGAGAATGCCCCTAA
GATCCTGCCCGGAAATGAGGAGCAGACATCAGGCACGCCCACGCAGCCCAAGACGTCTTGCTTAGCCACACCCCCAAGGG
AACTCAGCAGTAATAAACTTTAGACCATGAGTGTAAACTTGATCTAGTTATGGTTATAAGAGGGCCGGTAAAACTCGTGC
CAGCCACCGCGGTCATACGAGTGTTAGCCCAAGTGGATGGTTAACGGCGTAAAGAGTGGTTAGGGATCCCCAAAACTAAA
GCTGAACGCCCGCAGGGCCGTTATACGCATCCGAGAGCATGAAACCCCACCACGAAGGTGACTTTAACTTTACCCGAACC
CACGACAGCTAGGGTACAAACTGGGATTAGATACCCCACTATGCCTAGTCGTAAACTTTAATAGAAGCCGCACCCCCTCT
ATTCGCCAGGGCACTACGAGCACCAGCTTGAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTGTCC
TAGAACCGATACCCCCCGTTCAACCTCACCACTTCTAGCCCAAACCGCCTATATACCACCGTCGTCAGCTTACCCCGTGA
GGGACTAATAGTAAGCTAAACTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGCACGAAGTGGGAAGAGATGGGCTA
CATTTTCTGACCCAGAAAACTACGGAAGGAAAAATGAAAAGATTCCTGAAGGAGGATTTAGCAGTAAGCAGGGAGTAGAG
CGCCCCGCTGAAATAGGCCCTTAAGCGCGCACACACCGCCCGTCACTCTCCCCAAATCCTACCCTGCCATAACCTATAGC
CTTAAATGGATAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAAAACACAGTGTGTGGCTA
AATAGTAAAGCACCTCCCTTACACCGAGAAGATGTCCGTGCAATTCGGACCACCCTGATTCTAAAAAACTAGCCCGCCCC
TTAGAGAAACAACCCCCTCTAAATTATATTACCCGCACTACAAGCAACAAACCATTTACTTCCCCAGTACCCGGGCGACA
GAAAAG

Figure I. 18. 12S sequence of *Lampanyctus pusillus_4.*

>..\Sequences\SEQUENCING_2\B4+46af+Forward.ab1, ..\Sequences\SEQUENCING_2\F4+46a
AAGGCTTGGGTCCTGACTTTACTGTCGACTTTATCTAAACTTACACATGCAAGTCTCCGCCCTCCCGTGAGAATGCCCAT
AGTTTCCTGCTCGGAAACAAGGAGCTGGTATCAGGCACATCCTGTTGAGCCCACGACACCTTGCTTAGCCACACCCCCAA
GGGTACTCAGCAGTGATAAATATTAAGCCATAAGTGAAAACTTGACTTAATTACAGCTAAGAGGGCCGGTAAAACTCGTG
CCAGCCACCGCGGTTAGACGAGAGGCCCAAGTTGACAGACAACGGCGTAAAGAGTGGTTAAGGGCTGCAACAAACTAAAG
CTGAATGCTCTCCGGGCTGTCATACGTACCCGAGAGTTCGAAACCCAACTACGAAAGTGGCTTTATTAAACCCGACCCCA
CGAAAGCTAAGACACAAACTGGGATTAGATACCCCACTATGCTTAGCCCTAAACATCGATTTGATGACCCCATTAAATCC
GCCCGGAAATTACGAACATTAGTTTAAAACCCAAAGGACTTGGCGGTGCTTTATATCCACCTAGAGGAGCCTGTTCTAGA
ACCGATAATCCCCGTTAAACCTCACCCTCCCTTGCTCTACCCGCCTATATACCACCGTCGTCAGCTTACCCTGCAAAGGC
CCAATAGTAAGCAAAATTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGTGGATGGGAGGGGAAGAAATGGGCTACATT
TGCTAGCCATAGCAAATACGAATGATGCATTGAAACATGCAACTGAAGGAGGATTTAGTAGTAAGCAAAAATTAGAGTGT
TTAGCTGAAACCGGCCCTAAAGCGCGCACACACCGCCCGTCACCCTCCCCGAGCTACGAAGTACACATAACTAAAACCCT
ATAACTGCAAAGGGGAGGAAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAAAAAGCAGGATATAGCTAAGG
TAGTAAAGCACCCCCCTTACATCGAGGAGTCATCCGTGCAAGCCGGATTATCCTGACGCCCATCAGCTAGCCCCACCTTT
ATAATTAACAAATCCCTTTTATTTACCCTCAACCCCCCTCCCCAGATATAATTAAACCATTCTACCTCCTTAGTACGGGC
GACAGAAAAGAA

Figure I. 19. 12S sequence of *Lepidorhombus whiffiagonis_1.*

>..\Sequences\SEQUENCING_2\A7+48af+Forward.ab1, ..\Sequences\SEQUENCING_2\E7+48a
AGCACAAAGGCTTGGGTCCTGACTTTACTGTCGACTTTATCTAAACTTACACATGCAAGTCTCCGCCCTCCCGTGAGAAT
GCCCATAGTTTCCTGCTCGGAAACAAGGAGCTGGTATCAGGCACATCCTGTTGAGCCCACGACACCTTGCTTAGCCACAC
CCCCAAGGGTACTCAGCAGTGATAAATATTAAGCCATAAGTGAAAACTTGACTTAATTACAGCTAAGAGGGCCGGTAAAA
CTCGTGCCAGCCACCGCGGTTAGACGAGAGGCCCAAGTTGACAGACAACGGCGTAAAGAGTGGTTAAGGGCTGCAACAAA
CTAAAGCTGAATGCTCTCCGGGCTGTCATACGTACCCGAGAGTTCGAAACCCAACTACGAAAGTGGCTTTATTAAACCCG
ACCCCACGAAAGCTAAGACACAAACTGGGATTAGATACCCCACTATGCTTAGCCCTAAACATCGATTTGATGACCCCATT
AAATCCGCCCGGAAATTACGAACATTAGTTTAAAACCCAAAGGACTTGGCGGTGCTTTATATCCACCTAGAGGAGCCTGT
TCTAGAACCGATAATCCCCGTTAAACCTCACCCTCCCTTGCTCTACCCGCCTATATACCACCGTCGTCAGCTTACCCTGC
AAAGGCCCAATAGTAAGCAAAATTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGTGGATGGGAGGGGAAGAAATGGGC
TACATTTGCTAGCCATAGCAAATACGAATGATGCATTGAAACATGCAACTGAAGGAGGATTTAGTAGTAAGCAAAAATTA
GAGTGTTTAGCTGAAACCGGCCCTAAAGCGCGCACACACCGCCCGTCACCCTCCCCGAGCTACGAAGTACACATAACTAA
AACCCTATAACTGCAAAGGGGAGGAAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAAAAAGCAGGATATAG
CTAAGGTAGTAAAGCACCCCCCTTACATCGAGGAGTCATCCGTGCAAGCCGGATTATCCTGACGCCCATCAGCTAGCCCC
ACCTTTATAATTAACAAATCCCTTTTATTTACCCTCAACCCCCCTCCCCAGATATAATTAAACCATTCTACCTCCTTAGT
ACGGGCGACAGAAAAGAA

Figure I. 20. 12S sequence of *Lepidorhombus whiffiagonis_2.*

>..\Sequences\SEQUENCING_2\E9+45af+Forward.ab1, ..\Sequences\SEQUENCING_2\H9+45a
GCTTGGGTCCTGACTTTACTGTCGACTTTATCTAAACTTACACATGCAAGTCTCCGCCCTCCCGTGAGAATGCCCATAGT
TTCCTGCTCGGAAACAAGGAGCTGGTATCAGGCACATCCTGTTGAGCCCACGACACCTTGCTTAGCCACACCCCCAAGGG
TACTCAGCAGTGATAAATATTAAGCCATAAGTGAAAACTTGACTTAATTACAGCTAAGAGGGCCGGTAAAACTCGTGCCA
GCCACCGCGGTTAGACGAGAGGCCCAAGTTGACAGACAACGGCGTAAAGAGTGGTTAAGGGCTGCAACAAACTAAAGCTG
AATGCTCTCCGGGCTGTCATACGTACCCGAGAGTTCGAAACCCAACTACGAAAGTGGCTTTATTAAACCCGACCCCACGA
AAGCTAAGACACAAACTGGGATTAGATACCCCACTATGCTTAGCCCTAAACATCGATTTGATGACCCCATTAAATCCGCC
CGGAAATTACGAACATTAGTTTAAAACCCAAAGGACTTGGCGGTGCTTTATATCCACCTAGAGGAGCCTGTTCTAGAACC
GATAATCCCCGTTAAACCTCACCCTCCCTTGCTCTACCCGCCTATATACCACCGTCGTCAGCTTACCCTGCAAAGGCCCA
ATAGTAAGCAAAATTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGTGGATGGGAGGGGAAGAAATGGGCTACATTTGC
TAGCCATAGCAAATACGAATGATGCATTGAAACATGCAACTGAAGGAGGATTTAGTAGTAAGCAAAAATTAGAGTGTTTA
GCTGAAACCGGCCCTAAAGCGCGCACACACCGCCCGTCACCCTCCCCGAGCTACGAAGTACACATAACTAAAACCCTATA
ACTGCAAAGGGGAGGAAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAAAAAAGCAGGATATAGCTAAGGTA
GTAAAGCACCCCCCTTACATCGAGGAGTCATCCGTGCAAGCCGGATTATCCTGACGCCCATCAGCTAGCCCCACCTTTAT
AATTTAACAAATCCCCTTTTATTTACCCTCAACCCCCCTCCCCAGATATAATTAAACCATTCTACCTCCTTAGTACGGGC
GACAGAAAAGAAG

Figure I. 21. 12S sequence of *Lepidorhombus whiffiagonis_3.*

>..\Sequences\FOR.ab1\.ab1\E9+89+For.ab1, ..\Sequences\REV.ab1\.ab1\E9+89+Rev.ab
GGAAACGAGGAGCAAACATCAGGAACACATCTGTAGCCCATGACGTCTTGCGTGGCCACACCCCCAAGGGACTTCAGCAG
TAATAAACTTTAGGCAATAAGTGTAAACTTGACCTAGTCATGGTTATAAGAGAGCCGGTAAAACTCGTGCCAGCCACCGC
GGTCATACGAATGAGCCCAAGCGGATAATCGACGGCGTAAAGAGTGGTTAGGGGCCCCCAAAACTAAAGCTAAACGCTCG
CAAGGTCGTCGTACACATTCGATAGCATGAAACCCCACCACGAAAGTGGCTTTAACCCAACCCGAACCCACGATAGCTGG
GAAACAAACTGGGATTAGATACCCCACTATGCCCAGCCGTAAACATCAATAGAGACTATACAAACTTTATTCGCCCGGGA
ACTATGAGCATTAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTGTCCTAGAACCGATAA
CCCCCGTTCAACCTCACCGCTTCTTGCCAGACCCGCCTATATACCGCCGTCGTCAGCTTACCCCGTGAGGGGTCAGTAGT
AAGCTAAATTGGCACAGCCCAAAACGTCAGGCCGAGGTGTAGCACACGAAGTGTGGAAGAGATGGGCTACATTTTCTGCC
ACAGAAAATTACGAAGGGGGAAAGATGAAAAAACCCCCGAAGGAGGATTTAGCAGTAAGCGAGGAGCAGAGCGCCTCGCT
GAAGCCGGCCCTTTAGCGCGCACACACCGCCCGTCACCCTCCCCGAACCTACTCGCCAAGTAACATAACACGCCAACCGG
ACAAAGAGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAACAATACACACAGGGCGTGGCTAAACA
GCAAAGCACCTCACTTACACCGAGAAGATATCCGTGCAATTCGGATCGCCCTGATCTA

Figure I. 22. 12S sequence of *Lobianchia dofleini_1.*

>..\Sequences\FOR.ab1\.ab1\E11+91+For.ab1, ..\Sequences\REV.ab1\.ab1\E11+91+Rev.
AATGCCCCTAAAATCCCGCCCGGAAACGAGGAGCAGACATCAGGAACACATCTGTAGCCCATGACGTCTTGCGTGGCCAC
ACCCCCAAGGGACTTCAGCAGTAATAAACTTTAGGCAATAAGTGTAAACTTGACCTAGTCATGGTTATAAGAGAGCCGGT
AAAACTCGTGCCAGCCACCGCGGTCATACGAATGAGCCCAAGCGGATAATCGACGGCGTAAAGAGTGGTTAGGGGCCCCC
AAAACTAAAGCTAAACGCTCGCAAGGTCGTCGTACACATTCGATAGCATGAAACCCCACCACGAAAGTGGCTTTAACCCA
ACCCGAACCCACGATAGCTGGGAAACAAACTGGGATTAGATACCCCACTATGCCCAGCCGTAAACATCAATAGAGACTAT
ACAAACTTTATTCGCCCGGGAACTATGAGCATTAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGG
AGCCTGTCCTAGAACCGATAACCCCCGTTCAACCTCACCGCTTCTTGCCAGACCCGCCTATATACCGCCGTCGTCAGCTT
ACCCCGTGAGGGATCAGTAGTAAGCTAAATTGGCACAGCCCAAAACGTCAGGCCGAGGTGTAGCACACGAAGTGTGGAAG
AGATGGGCTACATTTTCTGCCACAGAAAATTACGAAGGGGGAAAGATGAAAAAACCCCCGAAGGAGGATTTAGCAGTAAG
CGAGGAGCAGAGCGCCTCGCTGAAGCCGGCCCTTTAGCGCGCACACACCGCCCGTCACCCTCCCCAAACCTACTCGCCAA
GTAACATAACACGCCAACCGGACAAAGAGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAACAATA
CACACAGGGTGTGGCTAAACAGCAAAGCACCTCACTTACACCGAGAAGATATCCGTGCAATTCGGATCGCCCTGATCCTA
AAAAGCTAGCCCAACCCACGCAGAAGCAACAAT

Figure I. 23. 12S sequence of *Lobianchia dofleini_2.*

>..\Sequences\FOR.ab1\.ab1\A2+2+For.ab1, ..\Sequences\REV.ab1\.ab1\A2+2+Rev.ab1
AAGGCACAAAGGCTTGGGTCCTGACTTTATTGTCAGCTCTAGCTAGACTTACACATGCAAGTCTCCGCACCCCTGTGAGA
ATGCCCGCGTCCCCTTATGAGGACTCGGAGCCGGTATCAGGCACCACCCCCATCCTTGCCCACGACGCCTTGCTTAGCCA
CACCCCTAAGGGAATTCAGCAGTGATAAATATTAAGCCATAAGTGAAAACTTGACTTAATTAAGGCCAAGAGAGCCGGTA
AAACTCGTGCCAGCCACCGCGGTGATACGAGAGGCTCAAGTTGACAAACACGGCACAAAGGGTGGTTAAGGACCCCTTAG
AAACTAAAGTCGAACACTTCCAAGGCTGTTATACGCATCCGAAAGCATGAAGCCCAACCACGAAAGTAACTTTAATACCC
CTGAACCCACGAAAGCTAAGAAACAAACTGGGATTAGATACCCCACTATGCTTAGCCGTAAACATCGATTTTTCCACAAA
CATCCGCCCGGGTACTACGAACACGAGTTTGAAACCCAAAGGACTTGGCGGTGCTTAACATCCACCTAGAGGAGCCTGTT
CTAGAACCGATAACCCCCGTTTAACCTCACCCTCCCTAGCCTAAAACCGCCTATATACCACCGTCGCCAGCTTACCCTGC
AAAGGCCCCACAGTAAGCAAAACTAGCACAGCTCAGTACGTCAGGTCGAGGTGTAGCGCATGAGAGGGGAAGAAATGGGC
TACATTCGCTACTTTAGCGTATAACGAATGCCTGCGTTGAAAAACGCATCTGAAGGAGGATTTAGAAGTAAGCAAAAAAT
AGAGTGTTTTGCTGAAACTTGGCACTAAAGCGCGCACATACCGCCCGTCACTCTCTCCCGTATATAACACATTAAAACCT
ATTTATAATACCACACCACAATTATTAGGAGAGGAAAGTCGTAACATGGTAAGCGTACTGGAAAGTGCGCTTGGGCAACC
AGAGTGTAGCTAAACAGTATAGCATCTCCCTTACACTGAGAAGTCATTCGTGCAAACCGAATCACCCTGATACTGAACAG
CTAGCCTAAACGTATCAAAAACAACAAACCACTATTAATAACCCCTAACACACCCCACACAAATAATTAAACCATTTTAC
CCTTAGTACGGGAGACGGAAAAGGACC

Figure I. 24. 12S sequence of *Microchirus variegatus.*

>..\Sequences\SEQUENCING_2\B1+76af+Forward.ab1, ..\Sequences\SEQUENCING_2\F1+76a
CCTGACTTTACTGTCAGCTTTAGCTAGATTTACACATGCAAGTATCCGCCTCCCTGTGAGAATGCCCATAGTGCCCTTTT
CGGGAACAAGGAGCTGGTATCAGGCACACGACCTACGTAGCCCACGACACCTTGCTTAGCCACACCCCCAAGGGAATCCA
GCAGTGATAAATATTAAGCCATAAGTGAAAACTTGACTTAGTTAAAGCTAAGAGAGCCGGTAAAACTCGTGCCAGCCACC
GCGGTTATACGAGAGGCTCAAGTTGACAGACAACGGCGTAAAGAGTGGTTAAGGAAAACATTCAACTAAAGCGGAACCCC
CTCACTGCTGTTATACGCTTCCGAGGGAATGAACCCCAACTACGAAGGTGGCTTTATATTAACCTGAACCCACGAAAGCT
AAGAAACAAACTGGGATTAGATACCCCACTATGCTTAGCCTTAAACATTGATTATTTATTACATCAAACATCCGCCCGGG
TATTACGAACATTAGTTTAAAACCCAAAGGACTTGGCGGTGCTTAACATCCACCTAGAGGAGCCTGTTCTAGAACCGATA
ACCCCCGTTCAACCTCACCCTCCCTAGCTTTTTCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGACTAATAG
TAAGCGCAATTGGTACAACCCAAAACGTCAGGCCGAGGTGTAGTGCATGAGAGGGGAAGAAATGGGCTACATTCGCTGCT
TATCAGCGAATAACGAATGATGCATTGAAATTATGCAGCTGAAGGAGGATTTAGCAGTAAGTGGAAAGTAGAGTGTTCCA
CTGAAACCGGCTCTTAAGCGCGCACACACCGCCCGTCACCCTCCCCAAGCTCCTGGACCCTAATCCTACTTAAACCCTAA
CAAATGCGAAGGAGAGGAAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCGCTTGGTAAAATCAGAGCGTAGCTAAAGT
AGAAAAGCATCTCCCTTACACTGAGAAGTCATCCGTGCAAGTCGGATCGCCCTGACGCCTATTAGCTAGCTCCGCCTACT
AAACCTCAACAAACAAACATAAATAAACCCAAAAGCACTAGACTGCAAATCAACCAAAGCATTTCCCTCCTCAGTATGGG
CGACAGAAAAGG

Figure I. 24. 12S sequence of *Mullus surmuletus_1.*

>..\Sequences\SEQUENCING_2\A12+77af+Forward.ab1, ..\Sequences\SEQUENCING_2\D11+7
TTGGGTCCTGACTTTACTGTCAGCTTTAGCTAGATTTACACATGCAAGTATCCGCCTCCCTGTGAGAATGCCCATAGTGC
CCTTTTCGGGAAACAAGGAGCTGGTATCAGGCACACGACCTACGTAGCCCACGACACCTTGCTTAGCCACACCCCCAAGG
GAATCCAGCAGTGATAAATATTAAGCCATAAGTGAAAACTTGACTTAGTTAAAGCTAAGAGAGCCGGTAAAACTCGTGCC
AGCCACCGCGGTTATACGAGAGGCTCAAGTTGACAGACAACGGCGTAAAGAGTGGTTAAGGAAAACATTCAACTAAAGCG
GAACCCCCTCACTGCTGTTATACGCTTCCGAGGGAATGAACCCCAACTACGAAGGTGGCTTTATATTAACCTGAACCCAC
GAAAGCTAAGAAACAAACTGGGATTAGATACCCCACTATGCTTAGCCTTAAACATTGATTATTTATTACATCAAACATCC
GCCCGGGTATTACGAACATTAGTTTAAAACCCAAAGGACTTGGCGGTGCTTAACATCCACCTAGAGGAGCCTGTTCTAGA
ACCGATAACCCCCGTTCAACCTCACCCTCCCTAGCTTTTTCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGA
CTAATAGTAAGCGCAATTGGTACAACCCAAAACGTCAGGCCGAGGTGTAGTGCATGAGAGGGGAAGAAATGGGCTACATT
CGCTGCTTATCAGCGAATAACGAATGATGCATTGAAATTATGCAGCTGAAGGAGGATTTAGCAGTAAGTGGAAAGTAGAG
TGTTCCACTGAAACCGGCTCTTAAGCGCGCACACACCGCCCGTCACCCTCCCCAAGCTCCTGGACCCTAATCCTACTTAA
ACCCTAACAAATGCGAAGGAGAAGGAAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCGCTTGGTAAAATCAGAGCGTA
GCTAAAGTAGAAAAGCATCTCCCTTACACTGAGAAGTCATCCGTGCAAGTCGGATCGCCCTGACGCCTATTAGCTAGCTC
CGCCTACTAAACTCAACAAACAAACATAAATAAACCCAAAAGCACTAGACTGCAAATCAACCAAAGCATTTCCCTCCTCA
GTATGGGCGA

Figure I. 25. 12S sequence of *Mullus surmuletus_2.*

>..\Sequences\SEQUENCING_2\B5+75af+Forward.ab1, ..\Sequences\SEQUENCING_2\F5+75a
TTGGGTCCTGACTTTACTGTCAGCTTTAGCTAGATTTACACATGCAAGTATCCGCCTCCCTGTGAGAATGCCCATAGTGC
CCTTTTCGGGAACAAGGAGCTGGTATCAGGCACACGACCTACGTAGCCCACGACACCTTGCTTAGCCACACCCCCAAGGG
AATCCAGCAGTGATAAATATTAAGCCATAAGTGAAAACTTGACTTAGTTAAAGCTAAGAGAGCCGGTAAAACTCGTGCCA
GCCACCGCGGTTATACGAGAGGCTCAAGTTGACAGACAACGGCGTAAAGAGTGGTTAAGGAAAACATTCAACTAAAGCGG
AACCCCCTCACTGCTGTTATACGCTTCCGAGGGAATGAACCCCAACTACGAAGGTGGCTTTATATTAACCTGAACCCACG
AAAGCTAAGAAACAAACTGGGATTAGATACCCCACTATGCTTAGCCTTAAACATTGATTATTTATTACATCAAACATCCG
CCCGGGTATTACGAACATTAGTTTAAAACCCAAAGGACTTGGCGGTGCTTAACATCCACCTAGAGGAGCCTGTTCTAGAA
CCGATAACCCCCGTTCAACCTCACCCTCCCTAGCTTTTTCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGAC
TAATAGTAAGCGCAATTGGTACAACCCAAAACGTCAGGCCGAGGTGTAGTGCATGAGAGGGGAAGAAATGGGCTACATTC
GCTGCTTATCAGCGAATAACGAATGATGCATTGAAATTATGCAGCTGAAGGAGGATTTAGCAGTAAGTGGAAAGTAGAGT
GTTCCACTGAAACCGGCTCTTAAGCGCGCACACACCGCCCGTCACCCTCCCCAAGCTCCTGGACCCTAATCCTACTTAAA
CCCTAACAAATGCGAAGGAGAGGAAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCGCTTGGTAAAATCAGAGCGTAGC
TAAAGTAGAAAAGCATCTCCCTTACACTGAGAAGTCATCCGTGCAAGTCGGATCGCCCTGACGCCTATTAGCTAGCTCCG
CCTACTAAACTCAACAAACAAACATAAATAAACCCAAAAGCACTAGACTGCAAATCAACCAAAGCATTTTCCCTCCTCAG
TATGGGCGACAGAAAAGGACCATG

Figure I. 26. 12S sequence of *Mullus surmuletus_3.*

>..\Sequences\FOR.ab1\.ab1\G2+113+For.ab1, ..\Sequences\REV.ab1\.ab1\G2+113+Rev.
GGTCCTGACTTTACTGTCTGCCCTAACCAAGACTATACATGCAAGTATCCGCACCCCCGTGAGAATGCCCTCAAAATCCC
GACCGGAAACGAGGAGCAGGCATCAGGCACACCCAACGCAGCCCAAGACGCCTTGCTTAGCCACACCCCCAAGGGAACTC
AGCAGTAATTAATATTAGGCAATAAGTGTAAACTTGACCTAACTATGGTTAAAAGGGCCGGTAAAACCCGTGCCAGCCAC
CGCGGTCATACGAGTGTTCGCCCAAGTGGACAGTTAGCGGCGTAAAGCGTGGTTAGGGAACCCAAAGTAACTAAAGAAAA
ACTACCTCCGGGCTGTTATACGCACCCGATAGTGTGAGACCCCATCACGAAAGTGACTTTAACTTGACCCTGAACCCACG
AAAGCCGGGAAACAAACCGGGATTAGATACCCCATTATGCCCAGCCGTAAACACAAATAGGTAACCTACTATACCTATTC
GCCCGGGAACTACAAGCATTAGCTTCAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTGTCCTAGA
ACCGATACCCCCCGTTCAACCTCACCACTTCTTGCTTAACCCGCCTATATACCACCGTCGTCAGCTTACCCTGTGAAGGA
TAAATAGTAAGCTAAATCGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGCACGAAGTGTGCAAGAGATGGGCTACAT
TTTCTGACCCAGAAAACTACGAAAGAAGAAATGAAAAACCTTCCGAAGGAGGATTTAGCAGTAATCAAGGAATAGAGCGC
CCTGATGAAACCGGCCCTTAAGCATGCACACACCGCCCGTCACTCTCCCCGAAGTCTCTCTACAGCCTTAACTTATAGCA
CAAAACTGACAAAGAGGAGGAAAGTCGTAACATGGTAAGTGTACCGGAAGGTGTACTTGGAATACACAGGGTGTGGCTAA
ACAGTAAAGCACCTCCCTTACACCGAGAAGACGTCTGTGCAATTCGGACCACCCTGAGCCTAAAAAGCTAGCCCCACCCC
CAAGAAAACAACCCCTTCTAAATTAGAACGCCACAAAACAAACAACAAAACATTTACCTCCCCAGTATGGGCGATAGAAA
AGGA

Figure I. 27. 12S sequence of *Myctophum puntctatum_1.*

>..\Sequences\FOR.ab1\.ab1\G3+114+For.ab1, ..\Sequences\REV.ab1\.ab1\G3+114+Rev.
AAAAGCCTGGGTCCTGACTTTACTGTCTGCCCTAACCAAGACTATACATGCAAGTATCCGCACCCCCGGTGAGAATGCCC
TCAAAATCCCGACCGGAAACGAGGAGCAGGCATCAGGCACACCCAACGCAGCCCAAGACGCCTTGCTTAGCCACACCCCC
AAGGGAACTCAGCAGTAATTAATATTAGGCAATAAGTGTAAACTTGACCTAACTATGGTTAAAAGGGCCGGTAAAACCCG
TGCCAGCCACCGCGGTCATACGAGTGTTCGCCCAAGTGGACAGTTAGCGGCGTAAAGCGTGGTTAGGGAACCCAAAGTAA
CTAAAGAAAAACTACCTCCGGGCTGTTATACGCACCCGATAGTGTGAGACCCCATCACGAAAGTGACTTTAACTTGACCC
TGAACCCACGAAAGCCGGGAAACAAACCGGGATTAGATACCCCATTATGCCCAGCCGTAAACACAAATAGGTAACCTACT
ATACCTATTCGCCCGGGAACTACAAGCATTAGCTTCAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGC
CTGTCCTAGAACCGATACCCCCCGTTCAACCTCACCACTTCTTGCTTAACCCGCCTATATACCACCGTCGTCAGCTTACC
CTGTGAAGGATAAATAGTAAGCTAAATCGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGCACGAAGTGTGCAAGAGA
TGGGCTACATTTTCTGACCCAGAAAACTACGAAAGAAGAAATGAAAAACCTTCCGAAGGAGGATTTAGCAGTAATCAAGG
AATAGAGCGCCCTGATGAAACCGGCCCTTAAGCATGCACACACCGCCCGTCACTCTCCCCGAAGTCTCTCTACAGCCTTA
ACTTATAGCACAAAACTGACAAAGAGGAGGAAAGTCGTAACATGGTAAGTGTACCGGAAGGTGTACTTGGAATACACAGG
GTGTGGCTAAACAGTAAAGCACCTCCCTTACACCGAGAAGACGTCTGTGCAATTCGGACCACCCTGAGCCTAAAAAGCTA
GCCCCACCCCCAAGAAAACAACCCCTTCTAAATTAGAACGCCACAAAACAAACAACAAAACATTTACCTCCCCAGTATGG
GCGATAGAAA

Figure I. 28. 12S sequence of *Myctophum puntctatum_2.*

>..\Sequences\FOR.ab1\.ab1\G4+115+For.ab1, ..\Sequences\REV.ab1\.ab1\G4+115+Rev.
AAAAGCCTGGGTCCTGACTTTACTGTCTGCCCTAACCAAGACTATACATGCAAGTATCCGCACCCCCGTGAGAATGCCCT
CAAAATCCCGACCGGAAACGAGGAGCAGGCATCAGGCACACCCAACGCAGCCCAAGACGCCTTGCTTAGCCACACCCCCA
AGGGAACTCAGCAGTAATTAATATTAGGCAATAAGTGTAAACTTGACCTAACTATGGTTAAAAGGGCCGGTAAAACCCGT
GCCAGCCACCGCGGTCATACGAGTGTTCGCCCAAGTGGACAGTTAGCGGCGTAAAGCGTGGTTAGGGAACCAAAAGTAAC
TAAAGAAAAACTACCTCCGGGCTGTTATACGCACCCGATAGTGTGAGACCCCATCACGAAAGTGACTTTAACTTGACCCT
GAACCCACGAAAGCCGGGAAACAAACCGGGATTAGATACCCCATTATGCCCAGCCGTAAACACAAATAGGTAACCTACTA
TACCTATTCGCCCGGGAACTACAAGCATTAGCTTCAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCC
TGTCCTAGAACCGATACCCCCCGTTCAACCTCACCACTTCTTGCTTAACCCGCCTATATACCACCGTCGTCAGCTTACCC
TGTGAAGGATAAATAGTAAGCTAAATCGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGCACGAAGTGTGCAAGAGAT
GGGCTACATTTTCTGACCCAGAAAACTACGAAAGAAGAAATGAAAAACCTTCCGAAGGAGGATTTAGCAGTAATCAAGGA
ATAGAGCGCCCTGATGAAACCGGCCCTTAAGCATGCACACACCGCCCGTCACTCTCCCCGAAGTCTCTCTACAGCCTTAA
CTTATAGCACAAAACTGACAAAGAGGAGGAAAGTCGTAACATGGTAAGTGTACCGGAAGGTGTACTTGGAATACACAGGG
TGTGGCTAAACAGTAAAGCACCTCCCTTACACCGAGAAGACGTCTGTGCAATTCGGACCACCCTGAGCCTAAAAAGCTAG
CCCCACCCCCAAGAAAACAACCCCTTCTAAATTAGAACACCACAAAACAAATAACAAAACATTTACCTCCCCAGTATGGG
CGATAGAAAAGGA

Figure I. 29. 12S sequence of *Myctophum puntctatum_3.*

>..\Sequences\FOR.ab1\.ab1\G5+116+For.ab1, ..\Sequences\REV.ab1\.ab1\G5+116+Rev.
AAAAGCCTCGAGAAGCACAAAAGCCTGGGTCCTGACTTTATTATCAGCCTTAACCTAACTTATACATGCAAGTATCCGCA
TCCCCGTGAGAATGCCCCTAAATCCTGCCCGGAAGCAAGGAGCAGGTATCAGGCACACTAACCGTAGCCCAAGACACCTT
GCTCAGCCACACCCCTAAGGGAACTCAGCAGTAATAAACATTAGGCAATAAGTGTAAACTTGACCTAGTCCCGGTTAATA
AGGGCCGGTAAAGCTCGTGCCAGCCACCGCGGTCATACGATGTTGTGCCCAAGCAGATAATCCACGGCGTAAAGTGTGGT
TAGGGAGACACTACAACTAAAGCTAAACGCTGACCAAGCCGTGATACGCATTTGGCAGCATGAAACCCCACCACGAAAGT
GGCTTTAAACAAACCTGAACCCACGAAAGCCAGGAAACAAACTGGGATTTGATACCCCACTATGCCTGGTCGTAAACAAT
GATAGACATCCACAGCGTCTATCCGCCTGGGAACTACGAGCCCCCCGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGA
CCCACCTAGAGGAGCCTGTCCTAGAACCGATACCCCCCGTTCAACCTCACCACTTCTAGCTCAATCCGCCTATATACCGC
CGTCGTCAGCTCACCCCGTGAGGGTCAAATAGTGAGCTTAATCGGCACAGCCCAGAACGTCAGGTCAAGGTGTAGCGCAC
GAAGTGTGGCAGAGATGGGCTACATTTTCTGACACAGAAAACCACAGATAGGATTCATGAAACCTTCCTTGAAGGAGGAT
TTAGCAGTAAGCAGGTAATAGAGTGACCCGCTGAAACCGGCCCTTAAGCGCGTACACACCGCCCGTCACTCTCCCCACAA
CTTAACCTTAGTACAACATAATGAACCAACAAGTATTCAGGGGAGGCAAGTCGTAACACGGTAAGCATACCTGAAGGTGC
GCTTGGATAACCCAGGGTGTGGCTAAATAGCAAAGCGCCTCCCTTACACCGAGAAGATGTCCGTGCAAATCGAACCACTC
TGATTCTAAAAAGCTAGCCCACCCCCTAAGAAATCAACCCCCTTTAAACCTAACTACCACAAAACAATAAACAAACCATT
TTCCCTCCCTAGTACATGGGCGACAAAAAAGG

Figure I. 30. 12S sequence of *Notolychnus valdiviae_1.*

>..\Sequences\FOR.ab1\.ab1\G6+117+For.ab1, ..\Sequences\REV.ab1\.ab1\G6+117+Rev.
AGCCTGGGTCCTGACTTTATTATCAGCCTTAACCTAACTTATACATGCAAGTATCCGCATCCCCGTGAGAATGCCCCTAA
ATCCTGCCCGGAAGCAAGGAGCAGGTATCAGGCACACTAACCGTAGCCCAAGACACCTTGCTCAGCCACACCCCTAAGGG
AACTCAGCAGTAATAAACATTAGGCAATAAGTGTAAACTTGACCTAGTCCCGGTTAATAAGGGCCGGTAAAGCTCGTGCC
AGCCACCGCGGTCATACGATGTTGTGCCCAAGCAGATAATCCACGGCGTAAAGTGTGGTTAGGGAGACACTACAACTAAA
GCTAAACGCTGACCAAGCCGTGATACGCATTTGGCAGCATGAAACCCCACCACGAAAGTGGCTTTAAACAAACCTGAACC
CACGAAAGCCAGGAAACAAACTGGGATTTGATACCCCACTATGCCTGGTCGTAAACAATGATAGACATCCACAGCGTCTA
TCCGCCTGGGAACTACGAGCCCCCCGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTGTCC
TAGAACCGATACCCCCCGTTCAACCTCACCACTTCTAGCTCAATCCGCCTATATACCGCCGTCGTCAGCTCACCCCGTGA
GGGTCAAATAGTGAGCTTAATCGGCACAGCCCAGAACGTCAGGTCAAGGTGTAGCGCACGAAGTGTGGCAGAGATGGGCT
ACATTTTCTGACACAGAAAACCACAGATAGGATTCATGAAACCTTCCTTGAAGGAGGATTTAGCAGTAAGCAGGTAATAG
AGTGACCCGCTGAAACCGGCCCTTAAGCGCGTACACACCGCCCGTCACTCTCCCCACAACTTAACCTTAGTACAACATAA
TGAACCAACAAGTATTCAGGGGAGGCAAGTCGTAACACGGTAAGCATACCTGAAGGTGCGCTTGGATAACCCAGGGTGTG
GCTAAATAGCAAAGCGCCTCCCTTACACCGAGAAGATGTCCGTGCAAATCGAACCACTCTGATTCTAAAAAGCTAGCCCA
CCCCCTAAGAAATCAACCCCCTTTAAACCTAACTACCACAAAACAATAAACAAACCATTTTCCCTCCCTAGTACATGGGC
GACAAAAAAG

Figure I. 31. 12S sequence of *Notolychnus valdiviae_2*

>..\Sequences\FOR.ab1\.ab1\G7+118+For.ab1, ..\Sequences\REV.ab1\.ab1\G7+118+Rev.
AAAGCCTGGGTCCTGACTTTATTATCAGCCTTAACCTAACTTATACATGCAAGTATCCGCATCCCCGTGAGAATGCCCCT
AAATCCTGCCCGGAAGCAAGGAGCAGGTATCAGGCACACTAACCGTAGCCCAAGACACCTTGCTCAGCCACACCCCTAAG
GGAACTCAGCAGTTAATAAACATTAGGCAATAAGTGTAAACTTGACCTAGTCCCGGTTAATAAGGGCCGGTAAAGCTCGT
GCCAGCCACCGCGGTCATACGATGTTGTGCCCAAGCAGATAATCCACGGCGTAAAGTGTGGTTAGGGAGACACTACAACT
AAAGCTAAACGCTGACCAAGCCGTGATACGCATTTGGCAGCATGAAACCCCACCACGAAAGTGGCTTTAAACAAACCTGA
ACCCACGAAAGCCAGGAAACAAACTGGGATTTGATACCCCACTATGCCTGGTCGTAAACAATGATAGACATCCACAGCGT
CTATCCGCCTGGGAACTACGAGCCCCCCGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTG
TCCTAGAACCGATACCCCCCGTTCAACCTCACCACTTCTAGCTCAATCCGCCTATATACCGCCGTCGTCAGCTCACCCCG
TGAGGGTCAAATAGTGAGCTTAATCGGCACAGCCCAGAACGTCAGGTCAAGGTGTAGCGCACGAAGTGTGGCAGAGATGG
GCTACATTTTCTGACACAGAAAACCACAGATAGGACTCATGAAACCTTCCTTGAAGGAGGATTTAGCAGTAAGCAGGTAA
TAGAGTGACCCGCTGAAACCGGCCCTTAAGCGCGTACACACCGCCCGTCACTCTCCCCACAACTTAACCTTAGTACAACA
TAATGAACCAACAAGTATTCAGGGGAGGCAAGTCGTAACACGGTAAGCATACCTGAAGGTGCGCTTGGATAACCCAGGGT
GTGGCTAAATAGCAAAGCGCCTCCCTTACACCGAGAAGATGTCCGTGCAAATCGAACCACTCTGATTCTAAAAAGCTAGC
CCACCCCCTAAGAAATCAACCCCCTTTAAACCTAACTACCACAAAACAATAAACAAACCATTTTCCCTCCCTAGTACATG
GGCGACAAAAAAGG

Figure I. 32. 12S sequence of *Notolychnus valdiviae_3.*

>..\Sequences\FOR.ab1\.ab1\G9+120+For.ab1, ..\Sequences\REV.ab1\.ab1\G9+120+Rev.
AAGCATAAAGGTTTGGGTCCTGACTTTGCTGTCGGCTCTGACTAGATTTACACATGCAAGTCTCCGCACCCCCGTGAGAA
TGCCCTTAGCCCCCCCACCGGGGGCAAGGAGCTGGTATCAGGCACGCACTGCAGCCCAAGACGCCTTGCTAAGCCACACC
CCCAAGGGAATTCAGCAGTGATAGATATTAAGCCATAAGCGAAAGCTTGACTTACTCAAGGTCAACATAGGGCTGGTAAA
TCTCGTGCCAGCCACCGCGGTTATACGAGCAAGCTCGAGTTGACAGGTGTCGGCGTAAAGGGTGGTCAGGGAAAGAACAA
CTAAAGTCGAACACCTCCTCAACTGTAATATGCGCCCGGAGGCATGAAGATCTACTACGAAAGTGGCTTTACTTTACTTG
AATCCACGACAGCTAAGAAACAAACTGGGATTAGATACCCCACTATGCTTAGCTATAAACTTTGATGTTAATGTACAACT
AGCATCCGCCAGGGGACTACAAGCACTAGCTTAAAACCCAAAGGACTTGGCGGTACCTTAGACCCCCCTAGAGGAGCCTG
TTCTAGAACCGATGATCCCCGTTGAACCTCACCACCCTTGGCTCAATTCTGTCTGTATACCGCCGTCGCCAGCTTACCCT
GTGAAGGATTTATAGTAAGCATAATGGGGATCCCCCAAAACGTCAGGTCGAGGTGCAGTGTATGGGGTGGGAAGAAATGG
GCTACATTTCCTAATGTAGGATACCACGAATATTGCTTTGAAATCAGCATAGAAGGTGGATTTAGCAGTAAGAAGGAAGC
AGAGAGTTCTTCTGAAGTTGGCTCTGAGGTGCGTACACACCGCCCGTCACCCTCCCCGCGTTAATCGTTTTGTCTAATTA
AGAAGTAAACCAAACAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGATTAAACAGGATGTG
GCTTAAGTAGAAAAGCATTTCTCTTACACTGAAAAGACACCCGTGAAACCCGGGTCGTCCTGAGCTGAATAGCTAGCCCA
ACAGATGAAGTGCCCGCAAGATGCCGACAAATGGCCGCACCTCAGCACGAAACTAAAAAATGTTAACAAACCATTTTCCA
TCTTAGTATAGGCGATAGAAAAGAA

Figure I. 33. 12S sequence of *Photostomias guernei_1.*

>..\Sequences\FOR.ab1\.ab1\G10+121+For.ab1, ..\Sequences\REV.ab1\.ab1\G10+121+Re
TCCGCAAGCATAAAGGTTTGGGTCCTGACTTTGCTGTCGGCTCTGACTAGATTTACACATGCAAGTCTCCGCACCCCCGT
GAGAATGCCCTTAGCCCCCCCACCGGGGGCAAGGAGCTGGTATCAGGCACGCACTGCAGCCCAAGACGCCTTGCTAAGCC
ACACCCCCAAGGGAATTCAGCAGTGATAGATATTAAGCCATAAGCGAAAGCTTGACTTACTCAAGGTCAACATAGGGCTG
GTAAATCTCGTGCCAGCCACCGCGGTTATACGAGCAAGCTCGAGTTGACAGGTGTCGGCGTAAAGGGTGGTCAGGGAAAG
AACAACTAAAGTCGAACACCTCCTCAACTGTAATATGCGCCCGGAGGCATGAAGATCTACTACGAAAGTGGCTTTACTTT
ACTTGAATCCACGACAGCTAAGAAACAAACTGGGATTAGATACCCCACTATGCTTAGCTATAAACTTTGATGTTAATGTA
CAACTAGCATCCGCCAGGGGACTACAAGCACTAGCTTAAAACCCAAAGGACTTGGCGGTACCTTAGACCCCCCTAGAGGA
GCCTGTTCTAGAACCGATGATCCCCGTTGAACCTCACCACCCTTGGCTTAATTCTGTCTGTATACCGCCGTCGCCAGCTT
ACCCTGTGAAGGATTTATAGTAAGCATAATGGGGATCCCCCAAAACGTCAGGTCGAGGTGCAGTGTATGGGGTGGGAAGA
AATGGGCTACATTTCCTAATGTAGGATACCACGAATATTGCTTTGAAATCAGCATAGAAGGTGGATTTAGCAGTAAGAAG
GAAGCAGAGAGTTCTTCTGAAGTTGGCTCTGAGGTGCGTACACACCGCCCGTCACCCTCCCCGCGTTAATCGTTTTGTCT
AATTAAGAAGTAAACCAAACAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGATTAAACAGG
ATGTGGCTTAAGTAGAAAAGCATTTCTCTTACACTGAAAAGACACCCGTGAAACCCGGGTCGTCCTGAGCTGAATAGCTA
GCCCAACAGATGAAGTGCCCGCAAGATGCCGACAAATGGCCGCACCTCAGCACGAAACTAAAAAATGTTAACAAACCATT
TTTCCATCTTAGTATAGGCGATAGAAAAGAA

Figure I. 34. 12S sequence of *Photostomias guernei_2.*

>..\Sequences\FOR.ab1\.ab1\G12+123+For.ab1, ..\Sequences\REV.ab1\.ab1\G12+123+Re
AGCATAAAGGTTTGGGTCCTGACTTTGCTGTCGGCTCTGACTAGATTTACACATGCAAGTCTCCGCACCCCCGTGAGAAT
GCCCTTAGCCCCCCACCGGGGGCAAGGAGCTGGTATCAGGCACGCACTGCAGCCCAAGACGCCTTGCTAAGCCACACCCC
CAAGGGAATTCAGCAGTGATAGATATTAAGCCATAAGCGAAAGCTTGACTTACTCAAGGTCAACATAGGGCTGGTAAATC
TCGTGCCAGCCACCGCGGTTATACGAGCAAGCTCGAGTTGACAGGTGTCGGCGTAAAGGGTGGTCAGGGAAAGAACAACT
AAAGTCGAACACCTCCCCAACTGTAATATGCGCCCGGAGGCATGAAGATCTACTACGAAAGTGGCTTTACTTTACTTGAA
TCCACGACAGCTAAGAAACAAACTGGGATTAGATACCCCACTATGCTTAGCTATAAACTTTGATGTTAATGTACAACTAG
CATCCGCCAGGGGACTACAAGCACTAGCTTAAAACCCAAAGGACTTGGCGGTACCTTAGACCCCCCTAGAGGAGCCTGTT
CTAGAACCGATGATCCCCGTTGAACCTCACCACCCTTGGCTTAATTCTGTCTGTATACCGCCGTCGCCAGCTTACCCTGT
GAAGGATTTATAGTAAGCATAATGGGGATCCCCCAAAACGTCAGGTCGAGGTGCAGTGTATGGGGTGGGAAGAAATGGGC
TACATTTCCTAATGTAGGATACCACGAATATTGCTTTGAAATCAGCATAGAAGGTGGATTTAGCAGTAAGAAGGAAGCAG
AGAGTTCTTCTGAAGTTGGCTCTGAGGTGCGTACACACCGCCCGTCACCCTCCCCGCGTTAATCGTTTTGTCTAATTAAG
AAGTGAGCCAAACAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGATTAAACAGGATGTGGC
TTAAGTAGAAAAGCATTTCTCTTACACTGAAAAGACACCCGTGAAACCCGGGTCGTCCTGAGCTGAATAGCTAGCCCAAC
AGATGAAGTGCCCGCAAGATGCCGACAAATGGCCGCACCTCAGCACGAAACTAAAAAATGTTAACAAACCATTTTTCCAT
CTTAGTATAGGCGATAGAAAAGAAT

Figure I. 35. 12S sequence of *Photostomias guernei_3.*

>..\Sequences\FOR.ab1\.ab1\B6+19+For.ab1, ..\Sequences\REV.ab1\.ab1\B6+19+Rev.ab
GCTTGGGTCCTGACTTTACTATCAACTCTAGCTAAACTTACACATGCAAGTATCCGCGACCCTGTGAGAATGCCCCACAG
TTTTCCGCCCGAAAACAAGGAGCTGGTATCAGGCACACCTAATAATAAAGCCCATGACGCCTTGCTTAGCCACACCCTCA
AGGGAACTCAGCAGTGATAAACCTTAAGCTATAAGTGCAAACTTGACTTAGTTAAAGCTAAGAGGGCCGGTAAAACTCGT
GCCAGCCACCGCGGTTATACGAGAGGCCCAAGTTGACAGACACCGGCGTAAAGCGTGGTTAAGGTAAATTAAAACTAAAG
CCGAACACCTTCAGGGCAGTTATACGCATCCGAAGGCACGAAGCCCCACCACGAAAGTGGCTTTATGATCCCTGACCCCA
CGAAAGCTATGACACAAACTGGGATTAGATACCCCACTATGCCTAGCCGTAAACATTGATAGAATTCTACACCCTCTATC
CGCCTGGGTACTACGAGCATTAGCTTGAAACCCAAAGGACTTGGCGGTACTTTAGATCCCCCTAGAGGAGCCTGTTCTAT
AACCGATGACCCCCGTTCAACCTCACCCTCCCTTGTTTATCCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGG
TCTAATAGTAAGCAAAATTGGCATCGCCCAGAACGTCAGGTCGAGGTGTAGCGCATGAGAGGGGAAGAAATGGGCTACAT
TCGCTAACATAGCGAATACGAACGATACACTGAAAACGTGTATCTGAAGGAGGATTTAGCAGTAAGCGGAAAATAGAGTG
TTCCGCTGAAATCGGCTCTGAAGTGCGTACACACCGCCCGTCACTCTCCCCAAGCTTATCAATACATATATCTAAAACGC
TTTAACCGCGAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAAAAATCAGAGTATAGCTAAG
ATAGAATAGCATTTCCCTTACACTGAAAAGTCATCCGTGCAAACCGGATTACCCTGACGCCGACCAGCTAGCCCACCCTA
ACAAAAGCAACAACCCAATATAAATAACCCCAAACACACAACTCCTCCTATAAACAAACCATTTTACCCCCCTAGTATGG
GCGACAGAAAAGGAAT

Figure I. 36. 12S sequence of *Sarda sarda.*

>..\Sequences\FOR.ab1\.ab1\A5+6+For.ab1, ..\Sequences\REV.ab1\.ab1\A5+6+Rev.ab1
AAAGCTTGGGTCCTGACTTTACTATCAACTTTAGCTATATTTACACATGCAAGTATCCGCACCCCTGTGAGAATGCCCTA
ATAGCTCCCCGCCCGGGAACAAGGAGCTGGTATCAGGCACAACCAACTGTAGCCCACGACACCTTGCTTTGCCACACCCT
CAAGGGAACTCAGCAGTGATAGACATTAAGCCATAAGTGAAAACTTGACTTAGTTAAAGCTAAGAGGGCCGGTTAAACTC
GTGCCAGCCACCGCGGTTATACGAGAGGCCCAAGTCGATAGTCAACGGCGTAAAGAGTGGTTAGAAGAAACCCATTACTA
AAGCCGAACGCCCTCAAAGCTGTTATACGCATCCGAAGGTGAGAAGCCCATCCACGAAAGTGGCTTTACAATCTTGAATC
CACGAAAGCTATGATACAAACTGGGATTAGATACCCCACTATGCTTAGCCCTAAACATTGACAACAACATACACCTGTTG
TCCGCCTGGGAACTACGAGCATCAGCTTGAAACCCAAAGGACTTGGCGGTGCTTTAGATCCACCTAGAGGAGCCTGTTCT
AGAACCGATAACCCCCGTTCAACCTCACCCTTCTTTGTTTCCCCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAA
GGATTAATAGTAAGCAAAATTGGTACAACCCTAAACGCCAGGTCGAGGTGTAGCGTATGGAAGGGGAAGAAATGGGCTAC
ATTCTCTAACACAGAGAAAACGAATGATGTACTGAAATACACGTCTGAAGGAGGATTTAGCAGTAAGCAGGAAATAGAGT
GTCCCGCTGAAATTGGCCCTGAAGCGCGCACACACCGCCCGTCACTCTCCCCAAACTAATTAAATTCAATTAAATAAAAC
CCCATCACAGTGAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAAAAATCAGGGCATAGCTA
AGACAGAAAAGCATCTCCCTTACACTGAGCAGTCATCCGTGCAAATCGGGTTGCCCTGACGCCCATTAGCTAGCCGTCTC
AACTAAAAACAACAAATCCCCATCAATACCCCCTAAGACACTCAAAACAACACTTAAACAAACCATTTTCCCCCCAAGTA
CGGGCGACAGAAAAGGAA

Figure I. 37. 12S sequence of *Umbria canariensis_1.*

>..\Sequences\FOR.ab1\.ab1\B1+14+For.ab1, ..\Sequences\REV.ab1\.ab1\B1+14+Rev.ab
CAAAAGCTTGGGTCCTGACTTTACTATCAACTTTAGCTATATTTACACATGCAAGTATCCGCACCCCTGTGAGAATGCCC
TAATAGCTCCCCGCCCGGGAACAAGGAGCTGGTATCAGGCACAACCAACTGTAGCCCACGACACCTTGCTTTGCCACACC
CTCAAGGGAACTCAGCAGTGATAGACATTAAGCCATAAGTGAAAACTTGACTTAGTTAAAGCTAAGAGGGCCGGTTAAAC
TCGTGCCAGCCACCGCGGTTATACGAGAGGCCCAAGTCGATAGTCAACGGCGTAAAGAGTGGTTAGAAGAAACCCATTAC
TAAAGCCGAACGCCCTCAAAGCTGTTATACGCATCCGAAGGTGAGAAGCCCATCCACGAAAGTGGCTTTACAATCTTGAA
TCCACGAAAGCTATGATACAAACTGGGATTAGATACCCCACTATGCTTAGCCCTAAACATTGACAACAACATACACCTGT
TGTCCGCCTGGGAACTACGAGCATCAGCTTGAAACCCAAAGGACTTGGCGGTGCTTTAGATCCACCTAGAGGAGCCTGTT
CTAGAACCGATAACCCCCGTTCAACCTCACCCTTCTTTGTTTCCCCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTG
AAGGATTAATAGTAAGCAAAATTGGTACAACCCTAAACGCCAGGTCGAGGTGTAGCGTATGGAAGGGGAAGAAATGGGCT
ACATTCTCTAACACAGAGAAAACGAATGATGTACTGAAATACACGTCTGAAGGAGGATTTAGCAGTAAGCAGGAAATAGA
GTGTCCCGCTGAAATTGGCCCTGAAGCGCGCACACACCGCCCGTCACTCTCCCCAAACTAATTAAATTCAATTAAATAAA
ACCCCATCACAGTGAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAAAAATCAGGGCATAGC
TAAGACAGAAAAGCATCTCCCTTACACTGAGCAGTCATCCGTGCAAATCGGGTTGCCCTGACGCCCATTAGCTAGCCGTC
TCAACTAAAAACAACAAATCCCCATCAATACCCCCTAAGACACTCAAAACAACACTAAACAAACCATTTTCCCCCCAAGT
ACGGGCGACAGAAAAGG

Figure I. 38. 12S sequence of *Umbria canariensis_2.*

>..\Sequences\SEQUENCING_2\B10+15r+Reverse.ab1, ..\Sequences\SEQUENCING_2\G9+15f
AAAAGCTTGGGTCCTGACTTTACTATCAACTTTAGCTATATTTACACATGCAAGTATCCGCACCCCTGTGAGAATGCCCT
AATAGCTCCCCGCCCGGGAACAAGGAGCTGGTATCAGGCACAACCAGTTGTAGCCCACGACACCTTGCTTTGCCACACCC
TCAAGGGAACTCAGCAGTGATAGACATTAAGCCATAAGTGAAAACTTGACTTAGTTAAAGCTAAGAGGGCCGGTAAAACT
CGTGCCAGCCACCGCGGTTATACGAGAGGCCCAAGTCGATAGTCAACGGCGTAAAGAGTGGTTAGAAAAAACCTGTTACT
AAAGCCGAACACCCTCAAAGCTGTTATACGCATCCGAAGGTGAGAAGCCCGTCCACGAAAGTGGCTTTACAATCTTGAAT
CCACGAAAGCTATGATACAAACTGGGATTAGATACCCCACTATGCTTAGCCCTAAACATTGACAACAACATACACCTGTT
GTCCGCCTGGGAACTACGAGCATCAGCTTGAAACCCAAAGGACTTGGCGGTGCTTTAGATCCACCTAGAGGAGCCTGTTC
TAGAACCGATAACCCCCGTTCAACCTCACCCTTCTTTGTTTCCCCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGA
AGGACTTATAGTAAGCAAAATTGGTACAACCCTAAACGCCAGGTCGAGGTGTAGCGTATGGAAGGGGAAGAAATGGGCTA
CATTCTCTAACATAGAGAAAACGAATGACGTACTGAAATACACGTCTGAAGGAGGATTTAGCAGTAAGCAGGAAATAGAG
TGTCCCGCTGAAATTGGCCCTGAAGCGCGCACACACCGCCCGTCACTCTCCCCAAACTAATTGAGTTCAATTAAATAAAA
CCCCATCACAGTAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAAAAATCAGGGCATAGCT
AAGACAGAAAAGCATCTCCCTTACACTGAGCAGTCATCCGTGCAAATCGGATTGCCCTGACGCCCACTAGCTAGCCGCCT
CCACTAAAAACAACAAATCCCCATCAATACCCCCTAATACACTCAAAACAACACTAAACAAACCATTTTTCCCCCTAAGT
ACGGGTGACAGAAAAGGAA

Figure I. 39. 12S sequence of *Umbria cirrosa.*

>..\Sequences\FOR.ab1\.ab1\H1+125+For.ab1, ..\Sequences\REV.ab1\.ab1\H1+125+Rev.
GAGAATGCCCTTAATTTCTCTTCCAGAAACGAGGAGCAAGCATCAGGCACGGGGAATAGCCCCAGCCCAAAACGCTTTGC
TAAGCCACCCCCCCAAGGGAATTCAGCAGTAATAAACATTAAGCTATGAGTGAAAGCTCGATCTAGTTAAAGTTAGGCGG
GTCGGTAAAACTCGTGCCAGCCACCGCGGTTATACGAGGGACCCTTGTTGATAATTGCCGGCGTAAAGAGTGGTTAAGGT
CCCCCCCATCTAAAGCTAAATACTTTCCCAGCTGTTATACGCCCTCGAAAAAAGACACCCTTTCACGAAAGTAGCTTTAC
CCTCCTGAACCCACTGCAATTAGGGAACAAACTAGGATTAGATACCCTACTATGCCTAATCATAAACTATGATATTAAAA
TACAACTAATATCCGCCAGGGTACTACGAGCACAGCTTAAAACCCAAAGGACTTGGCGGTACTTCAAACCCACCTAGAGG
AGCCTGTTCTAAAACCGATAATCCCCGTTTAACCTCACCCCTTCTAGTCAAGCCCGCTTATATACCGCCGTCGTCAGCCT
ACCCTGTAAAGGAACCCTAGTAAGCAAAAAAGACAAAACTCAAAAACGTCAGGTCGAGGTGTAGCGTATGAAGAGGGAAG
AAATGGGCTACATTTCCTGAATAAGGATACAACGGACAATATAATGAAATTTTTATTTGAAGGTGGATTTAGCAGTAAGA
AAAAACTAGCATGTTTTCCTGAAACCGGCTCTGAAGTGCGTACACACCGCCCGTCACTCTCCTCTATATACCCCACCTAG
TACATAAAACGCTAATTCTAATAAGGGGAGGCAAGTCGTAACACGGTAAGTGCACTGGAAAGTGCACTTGGAATAACCAT
GGCGCGGCTAACAAAGAAAAGCATTTCCCTTACACCGAAAAGACACCCGTGCAACTCGGGTCGCCCTGAACTAATAAGCT
AGCCACTTTCCTA

Figure I. 40. 12S sequence of *Vicinuerria attenuata_1.*

>..\Sequences\FOR.ab1\.ab1\H2+126+For.ab1, ..\Sequences\REV.ab1\.ab1\H2+126+Rev.
AAGGTTTGGGTCCTAGCTTTATTGTCAGCTTTAACTTTATTTATACATGCAAGTATCCGCCCCCCCGTGAGAATGCCCTT
AATTTCTCTTCCAGAAACGAGGAGCAAGCATCAGGCACGGGGAATAGCCCCAGCCCAAAACGCTTTGCTAAGCCACCCCC
CCAAGGGAATTCAGCAGTAATAAACATTAAGCTATGAGTGAAAGCTCGATCTAGTTAAAGTTAGGCGGGTCGGTAAAACT
CGTGCCAGCCACCGCGGTTATACGAGGGACCCTTGTTGATAATTGCCGGCGTAAAGAGTGGTTAAGGTCCCCCCCCATCT
AAAGCTAAATACTTTCCCAGCTGTTATACGCCCTCGAAAAAAGACACCCTTTCACGAAAGTAGCTTTACCCTCCTGAACC
CACTGCAATTAGGGAACAAACTAGGATTAGATACCCTACTATGCCTAATCATAAACTATGATATTAAAATACAACTAATA
TCCGCCAGGGTACTACGAGCACAGCTTAAAACCCAAAGGACTTGGCGGTACTTCAAACCCACCTAGAGGAGCCTGTTCTA
AAACCGATAATCCCCGTTTAACCTCACCCCTTCTAGTCAAGCCCGCTTATATACCGCCGTCGTCAGCCTACCCTGTAAAG
GAACCCTAGTAAGCAAAAAAGACAAAACTCAAAAACGTCAGGTCGAGGTGTAGCGTATGAAGAGGGAAGAAATGGGCTAC
ATTTCCTGAATAAGGATACAACGGACAATATAATGAAATTTTTATTTGAAGGTGGATTTAGCAGTAAGAAAAAACTAGCA
TGTTTTCCTGAAACCGGCTCTGAAGTGCGTACACACCGCCCGTCACTCTCCTCTATATACCCCCACCTAGTACATAAAAC
GCTAATTCTAATAAGGGGAGGCAAGTCGTAACACGGTAAGTGCACTGGAAAGTGCACTTGGAATAACCATGGGCGCGGCT
AACAAAGAAAAGCATTTCCCTTACACCGAAAAGACACCCCGTGCAACTCGGGTCGCCCTGAACTAATAAGCTAGCCACTT
TCCCTAAACTTTTAAACCTAATAAAACTTTAATAAAACCTATAACCCCTAAATAAACCATTCCCCCCTCTTAGTACAGGC
GACAAAAAA

Figure I. 41. 12S sequence of *Vicinuerria attenuata_2.*

>..\Sequences\FOR.ab1\.ab1\H4+128+For.ab1, ..\Sequences\REV.ab1\.ab1\H4+128+Rev.
AAAGGCTTGGGTCCCAGCTTTATTATCAGCTTTAACTTGATTTATACATGCAAGTATCTGCTCCCCCGTGAGAATGCCCT
TAATTTCTTTATCAGAAACGAGGAGCAAGCATCAGGCACGGACCCTCTTTCCAGCCCAAGACGCTTTGCTAAGCCACGCC
CTTAAGGGAATTCAGCAGTAATAAACATTAAGCCATGAACGAAAGCTCGACTTAGTTAAAGTTACCCGGGTCGGTAAAAC
TCGTGCCAGCCACCGCGGTTATACGAGAGACTCTTGTTGATAATTACCGGCGTAAAGCGTGGTTAAGGTCTGACTCACCT
AAAGCTAAACACTTTCCCAGCTGTTATACGCTCTCGAAAAAAGACACCCTTACACGAAAGTAGCTTTATCCTCCTGAACC
CACAACAATTAGGAAACAAACTAGGATTAGATACCCTACTATGCCTAATCATAAACTATGATATTAAAATACAACTAATA
TCCGCCAGGGTACTACGAGCACTAGCTTAAAACCCAAAGGACTTGGCGGTACTTCAAACCCACCTAGAGGAGCCTGTTCT
AGAACCGATAACCCACGTTTAACCTCACCACTTCTAGCCAAATCCGCTTATATACCGCCGTCGTCAGCTTACCCTATAAA
GGAACCTCAGTAAGCAAAAGAGACTAAAACTCAAAAACGTCAGGTCGAGGTGTAGCGTATGAAGTGGGAAGAAATGGGCT
ACATTTCCTACATTAAGGATACAACGAATAATAAAATGAAAATTTTTTTGAAGGTGGATTTAGCAGTAAGGAAGCCTAGC
ATATCTTCCTGAAACTGGCTCTGAAGTGCGTACACACCGCCCGTCACTCTCCCCTACTCACCCCTCTTAGTACATAAAAC
CCTATTTCTAATAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGAATAACCATGGCGCAGCTA
ACGAAGAAAAGCATTTCCCTTACACCGAAAAAGACACCCGTGCAACTCGGGTCGCCCTGAACTAACAAGCTAGCCGCTAT
TTCTAAACTTTACACCTAATGATTTATTTAATAAAACCCACAACCCCTAAACAAACCATTTTCCCCCTTAGTACAGGCGA
CAGAAAAGAAACTTAGAGCG

Figure I. 42. 12S sequence of *Vicinuerria nimbaria_1.*

>..\Sequences\FOR.ab1\.ab1\H5+130+For.ab1, ..\Sequences\REV.ab1\.ab1\H5+130+Rev.
TGGGTCCCAGCTTTATTATCAGCTTTAACTTGATTTATACATGCAAGTATCTGCTCCCCCGTGAGAATGCCCTTAATTTC
TTTATCAGAAACGAGGAGCAAGCATCAGGCACGGACCCTCTTTCCAGCCCAAGACGCTTTGCTAAGCCACGCCCTTAAGG
GAATTCAGCAGTAATAAACATTAAGCCATGAACGAAAGCTCGACTTAGTTAAAGTTACCCGGGTCGGTAAAACTCGTGCC
AGCCACCGCGGTTATACGAGAGACTCTTGTTGATAATTACCGGCGTAAAGCGTGGTTAAGGTCTGACTCACCTAAAGCTA
AACACTTTCCCAGCTGTTATACGCTCTCGAAAAAAGACACCCTTACACGAAAGTAGCTTTATCCTCCTGAACCCACAACA
ATTAGGAAACAAACTAGGATTAGATACCCTACTATGCCTAATCATAAACTATGATATTAAAATACAACTAATATCCGCCA
GGGTACTACGAGCACTAGCTTAAAACCCAAAGGACTTGGCGGTACTTCAAACCCACCTAGAGGAGCCTGTTCTAGAACCG
ATAACCCACGTTTAACCTCACCACTTCTAGCCAAATCCGCTTATATACCGCCGTCGTCAGCTTACCCTATAAAGGAACCT
CAGTAAGCAAAAGAGACTAAAACTCAAAAACGTCAGGTCGAGGTGTAGCGTATGAAGTGGGAAGAAATGGGCTACATTTC
CTACATTAAGGATACAACGAATAATAAAATGAAAATTTTTTTGAAGGTGGATTTAGCAGTAAGGAAGCCTAGCATATCTT
CCTGAAACTGGCTCTGAAGTGCGTACACACCGCCCGTCACTCTCCCCTACTCACCCCTCTTAGTACATAAAACCCTATTT
CTAATAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGAATAACCATGGCGCAGCTAACGAAGA
AAAGCATTTCCCTTACACCGAAAAGACACCCGTGCAACTCGGGTCGCCCTGAACTAACAAGCTAGCCGCTATTTCTAAAC
TTTACACCTAATGATTTATTTAATAAAACCCCAACCCCTAAACAAACCATTTTCCCCCCTAGTACAGGCGACAG

Figure I. 43. 12S sequence of *Vicinuerria nimbaria_2.*

>..\Sequences\FOR.ab1\.ab1\H6+131+For.ab1, ..\Sequences\REV.ab1\.ab1\H6+131+Rev.
GACTTTATTATCAGCTTTAACTTGATTTATACATGCAAGTATCTGCTCCCCCGTGAGAATGCCCTTAATTTCTTTATCAG
AAACGAGGAGCAAGCATCAGGCACGGACCCTCTTTCCAGCCCAAGACGCTTTGCTAAGCCACGCCCTTAAGGGAATTCAG
CAGTAATAAACATTAAGCCATGAACGAAAGCTCGACTTAGTTAAAGTTACCCGGGTCGGTAAAACTCGTGCCAGCCACCG
CGGTTATACGAGAGACTCTTGTTGATAATTACCGGCGTAAAGCGTGGTTAAGGTCTGACTCGCCTAAAGCTAAACACTTT
CCCAGCTGTTATACGCTCTCGAAAAAAGACACCCTTACACGAAAGTAGCTTTATCCTCCTGAACCCACAACAATTAGGAA
ACAAACTAGGATTAGATACCCTACTATGCCTAATCATAAACTATGATATTAAAATACAACTAATATCCGCCAGGGTACTA
CGAGCACTAGCTTAAAACCCAAAGGACTTGGCGGTACTTCAAACCCACCTAGAGGAGCCTGTTCTAGAACCGATAACCCA
CGTTTAACCTCACCACTTCTAGCCAAATCCGCTTATATACCGCCGTCGTCAGCTTACCCTATAAAGGAACCTCAGTAAGC
AAAAGAGACTAAAACTCAAAAACGTCAGGTCG

Figure I. 44. 12S sequence of *Vicinuerria nimbaria_3.*

>..\Sequences\FOR.ab1\.ab1\A1+1+For.ab1, ..\Sequences\REV.ab1\.ab1\A1+1+Rev.ab1
AAAGCTTGGGTCCTGACTTTACTATCAACTTTAGCTATATTTACACATGCAAGTATCCGCACCCCTGTGAGAATGCCCTA
ATAGCTCCCTGCCCGGGAACAAGGAGCTGGTATCAGGCACAACTAACTGTAGCCCACGACACCTTGCTTTGCCACACCCT
CAAGGGAACTCAGCAGTGATAGACATTAAGCCATAAGTGAAAACTTGACTTAGTTAAAGCTAAGAGGGCCGGTTAAACTC
GTGCCAGCCACCGCGGTTATACGAGAGGCCCAAGTCGATAGTCAACGGCGTAAAGAGTGGTTAGAAGGAGCCCATTACTA
AAGCCGAACACCCTCAAAGCTGTTATACGCACCCGAAGGTGAGAAGCCCATCCACGAAAGTGGCTTTACAACCTTGAATC
CACGAAAGCTATGATACAAACTGGGATTAGATACCCCACTATGCTTAGCCCTAAACATTGACAACAACATACACCTGTTG
TCCGCCTGGGAACTACGAGCATCAGCTTGAAACCCAAAGGACTTGGCGGTGCTTTAGATCCACCTAGAGGAGCCTGTTCT
AGAACCGATAACCCCCGTTCAACCTCACCCTTCTTTGTTTCCCCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAA
GGACTTATAGTAAGCAAAATTGGTACAACCCTAAACGCCAGGTCGAGGTGTAGCGTATGGAAGGGGAAGAAATGGGCTAC
ATTCTCTAACACAGAGAAAACGAATGATGTACTGAAATACACGTCTGAAGGAGGATTTAGCAGTAAGCAGGAAATATAGT
GTCCCGCTGAAATTGGCCCTGAAGCGCGCACACACCGCCCGTCACTCTCCCCAAATTAATTGAATTCAATTAAATAAAAC
CCCACCACAGTAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGAAAAATCAGGGCATAG

Figure I. 45. 12S sequence of *Zeus faber.*

>..\Sequences\FOR.ab1\.ab1\H7+136+For.ab1, ..\Sequences\REV.ab1\.ab1\H7+136+Rev.
CAAAGGCTTGGGTCCTGACTTTACTGTCAGCTTTAACCAGATTTACACATGCAAGTCTCCGCGCCCCTGTGAGGATGCCC
TCAGTCTCCCGCCCGGGAACGAGGAGCCGGCATCAGGCACAGCCCCGCAGCCCAAGACGCCTTGCTAAGCCACACCCCCA
AGGGAACTCAGCAGTGATAGATATTAAGCCATAAGCGAAAGCTTGACTTAGTTAAAGTTTATACAGGGCCGGTAAAACTC
GTGCCAGCCACCGCGGTTATACGAGAGGCCCAAGTTGACAGACACCGGCGTAAAGAGTGGTCAAGGGTATGAACAACTAA
AGCCGAACGTCCCCCTGGCTGTTATACGCACTCGGAGGAATGAAGCCCCACCACGAAAGTGGCTTTACCTAGCCTGAACC
CACGACAACTAAGAAACAAACTGGGATTAGATACCCCACTATGCTTAGCCGTAAACCTTGATACTAAGGTACAACTAGTA
TCCGCCAGGGGACTACAAGCGCCAGCTTAAAACCCAAAGGACTTGGCGGTACTTCAGACCCACCTAGAGGAGCCTGTTCT
AGAACCGATAACCCCCGTTTAACCTCACCACCCCTAGTTTTCCCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTAAA
GGCCCCACAGTAAGCAAAACGGGCATAGCCCAAAACGTCAGGTCGAGGTGTAGCGTATGAGGTGGAAAGAAATGGGCTAC
ATTTCCTAACTTAGGACACAACAGACGATGCCGTGAAACCAGCATCTGAAGGTGGATTTAGCAGTAAGAAGAAAGCAGAG
CGTTTTTCTGAAGCCGGCTCTGAGATGCGTACACACCGCCCGTCACTCTCCCCAAGTTCACCTCTATAAGTAAATAAAAG
AACAATAGAACTAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGAACAACCAGAGCGTAGCTT
AGTGAGTAGAGCACTTCCCTTACACCGAAGAGACACCCGTGCAAACCGAGTCGCCCTGAGCTGATCAGCTAGCCCAACAC
CCCTAGGTTCCCCTAACAACACACCCACCCTAACAAAACCTTGAAACCCCAAACAAACCATTTTTCCATCATAGTATGGG
AGACAGAAAAGA

Figure I. 46. 12S sequence of *Stomias.*